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SOME RECENT DEVELOPMENTS IN VIRUS RESEARCH

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ADDRESS OF THE RETIRING PRESIDENT OF THE ASSOCIATION OF APPLIED BIOLOGISTS, DELIVERED TO THE ANNUAL GENERAL MEETING ON FRIDAY, 11 FEBRUARY 1938

I PROPOSE to-day to discuss only two aspects of virus disease. One is the thoroughly practical problem of control, the other the highly abstract problem of the nature of a virus. To both problems noteworthy contributions have been made in the last two or three years, and it is my purpose to outline for you this progress. I shall not offer any original contributions of my own, but I do not think I need make any apology on that account. It is almost impossible for anyone not a specialist to keep up to date with the output of virus literature, and even for the specialist it is not easy: the mass is so overwhelming. When I was confined to bed at the beginning of the year, reprints kept piling up on my desk at the rate of more than one a day; and it is a new and interesting test of the state of one's health when the thought of this accumulation ceases to be a depressant and becomes a stimulant. The switch-over may be taken as the beginning of convalescence, and I suppose one might take as a sign of exuberant health the day when one was really thrilled by the description of a new virus disease in the potato plant. If that is so I am still below par—and have been for some years.

To such an audience as I have to-day I need hardly insist on the economic importance of virus disease at the present time. You must all of you have had personal experience of the damage it causes. We are passing at present through an outbreak of foot and mouth disease, and the daily press has been filled with estimates of the financial loss this outbreak has caused and is still causing to the country. But every year virus disease in plants produces losses on a similar scale, not only in this country but all over the world. It attracts little popular attention because it is so normal. It is rather like the death roll on the roads: we are too accustomed to it. Attempts are made to reduce it but, as in the other case, with only indifferent success. And yet the urgency of the question needs no emphasis.

It is not easy to get any precise quantitative estimate of the loss in any one crop due to virus disease; and quite impossible to make a worthwhile estimate of the aggregate loss in this country or the world as a whole. We know that, in sugar cane, mosaic disease some years ago was so serious as to threaten the destruction of the industry in Louisiana and the West Indies. In sugar beet the incidence of the curly-top disease varies greatly from year to year, largely according to the magnitude of the insect infestation. In a bad year the average yield per acre may fall from the normal of 11–15 tons to 1 or $1\frac{1}{2}$ tons; but, taking an average over a number of years, bad and less bad, the loss on the 763,000 acres in the western states of America is reckoned at about two million tons a year. In this country, in the potato crop virus costs about two million pounds a year. And this is one of the few cases where the estimate may be accepted as reasonably accurate. Every year about 500,000 acres are planted with potatoes in England and Wales. Of these 500,000 acres about 120,000 are planted with fresh seed tubers. The remaining 380,000 acres are planted with tubers from locally grown plants. We know that these local tubers contain a certain amount of virus, which increases progressively the longer the local stock has been in use. On the average replacing the local tubers by fresh seed raises the yield by 1 ton per acre. At the low valuation of £5 per ton that represents an annual loss in the 380,000 acres not sown with fresh seed of about two million pounds in the potato crop of England and Wales due to virus disease.

I quote these figures merely as examples to show the magnitude of the problem. If we could add to the potato losses of this country those in the glasshouse industries, in strawberries, raspberries and other small fruits, in hops, bulbs and in the flower trades, the figures would be very large indeed, even for England and Wales, and almost incredible if we could assess the loss throughout the world.

The whole problem of control falls into two main subdivisions: (1) the sowing of clean material; (2) the keeping the crop clean during the period of growth.

In the case of crops which are grown from true seed, the first is in the majority of instances fairly easy, because it is only in a few cases that virus is carried through the true seed. It happens in some of the Leguminosae and in one or two other cases. Whether it occurs in tomatoes and cucumbers, as has been maintained in this country, is still a matter of dispute. It is not really a very easy thing to settle, because it is notoriously difficult to prove a negative. There is a lot of evidence against it and some for it. Myself, I should not be inclined to be dogmatic either

way, because I am not sure that the disputing parties are always talking about the same things. It is at least possible that the evidence against is based on work with different viruses from that in favour. But, whatever the truth may eventually turn out to be in that particular question, it is not a very difficult matter to ensure that in crops grown from true seed the seed sown shall be free from disease.

But in the case of crops vegetatively propagated—that is to say, from tubers, bulbs, sets as in sugar cane, or suckers as in raspberries, and so on—the problem of starting with clean material is very much more difficult. One cannot without elaborate testing be sure that the plant from which one takes the tubers or bulbs or cuttings is in fact free from infection, even although it shows no external evidence of disease. It may have been infected only a short time before the propagating material was taken and contain the disease although not showing it. Or it may be in a carrying condition, either as a permanent carrier which never shows any symptoms at any time, or as a temporary carrier which shows symptoms for only a short time and then loses all visible signs.

This difficulty has given rise to elaborate methods of investigation of the plants which are to be used as the parents of the new crop. In this country you are familiar with the system of inspection of the growing potato crop, and the issue of certificates that the crops inspected contain only a specified small percentage of virus disease. In America there is an extensive system of tuber indexing, which is in effect a preliminary testing for the presence of virus before the issue of the tubers for planting.

These methods work out on the whole with a fair amount of success. There are, of course, cases where the inspection and certificates are misleading. This is not always due to fault on the part of the inspectors. The inspection is a visual one, and the potato crop in particular is liable to a masking of symptoms, which is dependent on the weather. After a bright sunny spell symptoms are much less evident than after a cloudy, wet, muggy period, and a crop may seem fairly free from disease at the times of inspection, when it is actually widely infected. But on the whole it works fairly satisfactorily, and there is a considerable probability that certificated seed is comparatively free from virus.

So that both with crops that are grown from true seed and crops that are vegetatively reproduced there is a reasonable chance that one is starting with comparatively clean material.

But it is a very different matter once the seed is planted and the crop is growing. It is then exposed to infection over a period of weeks or

months, and it is impossible to prevent this exposure by any practicable means known at the present time. The sources from which the infection may come are manifold. There is the source within the crop itself—in potatoes, for example, the small percentage of diseased plants among the parents supplying the tubers used as seed. Or it may be residual plants left in the ground from a previous crop. This is a frequent source of contamination. In the Sudan, for example, the incidence of leaf-curl in cotton came down astonishingly when an efficient means was devised of getting rid of the roots of the previous crop. In the tobacco industry the influence of residues and the effect of soil composition on the duration of infectivity of such residues have been demonstrated by James Johnson. In this country we have the ground keepers left after the harvest of the potato crop. These supply foci of infection within the crop itself. In addition there are the external sources: infected plants within carrying range, either plants of the same species or biennial or perennial weeds, which have been shown many times to be a reservoir for carrying over the infection through the winter. There is no reason to believe that insects themselves can to any significant extent carry over the disease from one season to the next. Those which are responsible for the bulk of virus spread do not commonly survive a winter in the adult stage, and there is only one authenticated case where infection has been shown to be transmitted through the egg. There is no difficulty in finding explanations, valid at least in theory, for the entry of a variable number of infections into the growing crop.

Every point of entry is a source of spread. There is no doubt that in most cases the agency of this spread is insect carriage. There are, of course, other mechanisms, which are sometimes operative. In diseases transmissible by juice the rubbing of an infected plant against a healthy one, especially under the action of wind, may transmit infection. You will have noticed a recent letter in *Nature* in which Prof. Murphy demonstrated that this occurs in potato with the virus X; and the same thing has been noted in tobacco. But this can be responsible for only a small amount of spread. It is too slow a process to be a very serious danger, and the scattered incidence of the disease over a growing crop shows that there are other more effective means. There is no satisfactory evidence of transmission through the air; or—except in one disease of wheat not known in this country—through the soil, apart from the presence of infective residues. There are some crops where mechanical transmission does play a very important part, namely, those in which the individual plants are handled by the cultivators. The stripping of the leaves in the

tobacco crop and the stopping out in tomatoes are examples. In such cases disease is spread broadcast on the hands of the operatives, and the juice of an infected plant carried to healthy plants widely through the crop. But these are special cases. In most instances the diseases are spread throughout a growing crop by the agency of insects.

This is very clearly seen in the curly-top disease of sugar beet in the United States. The virus has a very extensive host range, and its hosts include many of the plants on which the vector, *Eutettix tenellus*, a jassid, lives. In the spring the vector develops to an enormous extent on the weeds of the foothills or uncultivated plains, and when this vegetation begins to dry up as the season advances, the insects migrate to the cultivated areas and attack the sugar beet, returning in the autumn to the areas from which they came, ready to descend once more in the following season. The damage to the crop is closely correlated with the magnitude of the insect invasion. In years when the insects come in relatively small numbers, the damage from curly top is small; but in a bad insect year the virus invasion is enormous, and, as we have seen, the yield may fall from 11–15 tons an acre which is the normal down to 1 or $1\frac{1}{2}$ tons.

What determines the size of the insect invasion is still obscure—at least in detail. Climatic factors are obviously largely responsible, and it seems clear that unusually cold winters and early springs reduce the numbers. We have still a lot to learn about the variation in insect numbers from year to year, and the death of Maldwyn Davis, a member of our Association, has been a serious loss to virus entomology, because that was the type of problem which he was attacking with marked success.

There are, of course, cases in which the vector is unknown. The transmission of tobacco mosaic, for example, is one of the most puzzling problems to the plant pathologist at the present time. Here we have one of the most infectious diseases known, transmissible by the tiniest possible drop of infected juice, and yet of the many insects that feed upon the tobacco plant none is known to carry the disease, although one might have expected that of all diseases this would have been the most readily carried. In our own country virus X is one of the two most important sources of disease in the potato crop; but its vector—whose existence no one, I imagine, really doubts—is still unknown.

The practical problem of control, then, reduces to the questions—how are we to limit the invasion of the growing crop, and how are we to limit the spread from those points where entry has been gained?

The direct and finally most satisfactory method would be to interrupt the transmission by control of the insect. But as yet no practicable method of doing so has been devised. In limited areas and in special crops of high financial value, it may be possible by repeated spraying to reduce the incidence, as was shown by Mrs Watson for the small *Hyoscyamus* crop grown for medicinal purposes. But such a method is not practicable for large crops, such as potato, because of the expense. One might conceivably introduce into the ground some cheap chemical which would make the plants distasteful to the insect, but this has never so far as I know been suggested or tried. As yet no success has been obtained in interference with the insect.

Until recently the only general method that has had any real success has been the development of immune varieties. This has been conspicuously successful in the case of the sugar cane. Races have been developed—originally by the Dutch in Java—which give a high yield of sugar and are highly resistant to the mosaic which is the principal danger to the crop; and these strains are now grown throughout the world whereever the sugar cane is grown. There are, of course, recurrent difficulties. A strain which is immune to the virus of one locality may not be immune to the virus of another locality. You will remember the similar case of Biffen's rust-immune wheats. And even in the one locality a new strain of virus may appear, to which immunity has not been obtained, as has indeed happened in Louisiana. When this occurs, a new genetical complex must be arranged to meet the new conditions; and this takes time and it is not every industry that can bear the expense. But, undoubtedly, immune varieties have largely solved the sugar-cane problem. In other crops the success has not been so great. In the Sudan a strain of cotton has been obtained which is highly resistant to leaf curl and gives a fairly satisfactory product. In sugar beet, as the result of long and very costly investigations, some strains have been evolved which give a fair yield and, while not immune, have enough measure of resistance to make them commercially useful; and no doubt these strains will be further perfected. But apart from these crops the use of immune varieties has not been particularly successful. In this country, for example, we have no resistant varieties in our main virus-affected crops, and progress along this line has been very small.

In the last few years, however, a new method has been devised. It is still in the experimental stage, and it is not yet possible to estimate how successful it will be; but it is very promising. It has been found that the presence of a virus systemically distributed throughout a plant protects

that plant against subsequent invasion by certain other viruses. The fact was first described by Thung in Java, who was working with the diseases of tobacco; but his paper escaped the attention of any but the Dutch workers, and its possible practical significance was not at first appreciated. It was discovered independently by Salaman in Cambridge, who was working with potato viruses, and shortly afterwards was described by Kunkel in America, and almost simultaneously by Caldwell in this country, both of whom worked with diseases affecting tobacco and tomato. All these workers arrived apparently quite independently at the same results, but so far as I know it was Salaman who first realized the practical significance. Since these first reports a considerable amount of work has been devoted to the phenomenon, and a number of further facts established. It was soon shown that the protection conferred was in some sort specific. It did not hold against all viruses—that, of course, was evident from what we already knew about mixed infections—but only against viruses related to the original protection-conferring virus. Infection with potato virus *X* or *Y*, for example, gave no protection against subsequent infection with tobacco mosaic; nor preliminary infection with tobacco mosaic protection against *X* or *Y*. But infection with ordinary tobacco mosaic protected against later infection with aucuba or yellow mosaic, a more virulent strain of tobacco mosaic; and infection with a weak strain of *X* protected against later infection with a virulent strain. There is no need for the first strain to be a weak one: one virulent strain will protect against another related virulent strain. It is, however, not always easy to demonstrate the protection in the case of virulent strains, because the symptoms of the one strain mask or obscure the symptoms of the other.

The nature of this protection is still obscure. It would seem that the presence of the first strain does not prevent the entry or dissemination of the second, but it prevents it from exerting its normal destructive action. The first strain, wherever it establishes itself, blocks, as it were, the second strain, occupying the available ground, so that when the second strain arrives it finds a squatter already there and no opportunity for establishing itself, except in occasional areas that have escaped the first. A certain amount of time is necessary for the first strain to occupy the ground, and this period varies with the respective aggressiveness of the two strains: it may require a few days or several weeks. If the second strain is inoculated at the same time as the first, or before the necessary interval has elapsed, there is either no protection or a mixed infection results, in which the more virulent strain gradually dominates the other

and may oust it entirely in the later growth of the plant. But if the necessary interval has elapsed the second strain makes no headway, and apparently—though I am not sure of this—eventually dies out.

Thung has examined a considerable number of tobacco diseases from this point of view and has grouped them according to their penetrative powers and their dominance with respect to one another. But I need not go into details here, especially as he has not always examined the relationships of the strains to one another. The salient fact with which I am here concerned is that it is possible in some cases and perhaps in most to confer protection against a severe disease by a previous infection with another allied strain which may be of quite a mild type.

Now from the commercial point of view the grower does not care whether his crop is diseased or not, so long as the disease does not affect adversely either the quality or the quantity of the crop. And this is a not infrequent happening. There are some strains of tobacco mosaic known, for example, which produce merely a quite trivial and sometimes indistinguishable discoloration of the leaves. In potatoes some of our standard varieties are 100 % infected with a mild form of mosaic that reduces neither the number nor the quality of the tubers. In America practically every potato plant contains a latent virus which, in suitable varieties, has no perceptible effects.

Here then is a possibility of a practical commercial protection against severe disease. If one can infect in advance one's crop with a mild form of the common serious disease, one has protected it against that serious disease, and it does not matter how much it may be exposed to it during the period of growth. The crop is diseased, but its disease does not affect its commercial value.

This possibility is in course of trial at the present time. In Holland, Prof. Quanjer is carrying out extensive investigations on potatoes and with results that are, so far, most encouraging. In Cambridge, Salaman is also investigating the possibilities, and also with promising results. There is necessary, of course, a lot of preliminary research to discover suitable weak strains which shall give satisfactory protection without themselves causing significant damage; and he has now got strains both of X and Y, which are effective in laboratory conditions and are being tried out on the field scale. Only experience will show whether the protection will persist through several generations, whether it will be possible to combine in the one plant several protective infections against several of the severe types, whether the principle is applicable to all sorts of plants, and so on. But one may say that a new principle has been

discovered which may solve in a practical manner some part at least of the problem of control.

The method you will notice is essentially a vaccination. Now it is obviously impracticable to inoculate every plant in a standing crop of many acres. One can infect a certain number of foci and leave it to the natural means to spread the infection throughout the crop, hoping that this early infection will get to work before the severe type comes along. That perhaps is what one may have to do in the case of crops grown from true seed—at least those crops where virus is not transmitted through the true seed. But in the case of vegetatively reproduced crops the protection may be made almost universal. One can ensure that the parents from which one's propagating material is taken are already protectively infected, and it may be possible to build up races and stocks of plants immune to the graver types of disease, all of them infected but with infections that are not of practical significance to the grower. And as these races get spread throughout the country, the destructive types of disease may be gradually eliminated altogether.

The endeavour to obtain control, therefore, has taken a definite and a hopeful step forward in the last year or two.

I turn now to a very different subject, namely, the nature of a virus. As you know, this has been a subject of debate since the first discovery of the existence of viruses. In almost the first detailed account of the virus of tobacco mosaic Beijerinck described it as a *contagium fluidum vivum*, and since then there has been a continual interchange of amenities sometimes metaphysical, sometimes almost mystical, between the living and the non-living schools. On the one hand it was maintained that they were simply small organisms, no doubt with special properties because of their demonstrably small size and the consequent great increase of surface relatively to their bulk, but still essentially organisms analogous to small bacteria. On the other it was maintained that they could not be organisms in any ordinary usage of the term, that they were essentially non-living, and that, although they could not really be like the enzymes with which we were already familiar, they were autocatalytic complexes more akin to enzymes than to any autonomous creature.

The difference of opinion persisted because there was nothing known which was decisive one way or the other; and the view adopted probably depended on the way the disputant had been educated. If he was a chemist he naturally inclined to the non-organismal view; if a biologist to the small bacterium theory, and both were happy and argumentative. On the whole, I think the balance of opinion was, for many years at least,

definitely in favour of the organismal view. Since viruses could not be grown on cell-free media, they were held to be obligate parasites, and the fact that they required their food to be prepared for them in some way by the host was correlated with the smallness of their size. Apart from that condition of their existence, they were organisms as much as any other organism. They varied in size in a descending scale from comparatively large bodies to bodies which could be rendered visible only by photographing them with short wave-length light; and they continued to look like organisms in the photographs as each successive refinement of the photographer's art, each progressive reduction of the wave-length used, enabled one to penetrate further and further down the scale.

But, as research progressed, the organismal theory became gradually less convincing. It was shown by filtration through collodion membranes that some at least of the viruses were smaller in size than single molecules of known proteins such as the haemocyanins, and Laidlaw found a sewage organism which grew well in cell free media and yet was of similar size to many accepted viruses. It was difficult to believe that a thing the size of a single molecule could possess all the properties of life; and it began to be realized that no one could say what they meant by an organism, nor define precisely what is the difference between a thing that is living and one that is non-living.

Then came Stanley with his claim that he had isolated a crystalline protein with all the properties of tobacco mosaic virus. Now there was nothing new to the plant pathologist in the idea that virus is associated with crystals. Iwanowski pointed out thirty years ago that tobacco mosaic disease was characterized by the appearance in the cells of flat plate-like crystals not found in normal cells and exhibiting cross striation under the action of acid. Very much later, about 1931, when attempts were being made to obtain virus in a purified state, free from the non-specific ingredients of the plant, the idea was mooted that the virus might itself be crystalline. True crystals which had infective properties were in fact actually obtained, and the conception of a crystalline virus became familiar. But these crystals were soon shown to be really crystals of the materials used in the process of purification, and their infectivity was due to virus entangled as they formed. It was not till Stanley's paper came out that the possibility of a genuine crystalline virus had to be seriously entertained.

The assertion that the virus was crystalline was responsible for much of the attention at first given to the new claim, for those of us who had been brought up in the older order of ideas attached a kind of sanctity to

the word "crystal". It was a sort of guarantee of purity, and Stanley himself cited as evidence of the purity of his protein that it could be recrystallized as many as 15 times without change of properties. As a matter of fact, repeated recrystallization, at least in the case of large molecule proteins, gives no such guarantee of purity, and in the particular case of the crystalline material of Stanley, obtained by precipitation by ammonium sulphate, it has been shown by Bawden that it is demonstrably impure and retains its impurity, however many times it is reprecipitated. Moreover, it has been shown by Bernal that these so-called crystals of tobacco mosaic are not really crystals in the full sense of the word. They have some of the orderly molecular arrangement found in crystals, but so have many other things not usually described as crystalline, such as muscle fibres or hair structures. They have only a two-dimensional regularity instead of a three-dimensional, and they are better described as fibres or paracrystals. Actually it is a matter of secondary importance whether the protein as found appears to be fully crystalline or not. The observed form may be determined by the conditions under which the isolation is effected, and if these conditions were suitably modified, full three-dimensional crystals might result instead of the incomplete two-dimensional form. Even as it is, the protein can under certain conditions be obtained as long mesomorphic fibrils visible to the naked eye. But undoubtedly the crystalline property attributed to tobacco mosaic caught the attention of many who might otherwise have been slow to appreciate the significance of the new step.

At the same time as Stanley was working on tobacco mosaic, Bawden & Pirie in Cambridge had been isolating virus proteins from potato mosaic. The potato mosaic viruses do not give, or at least have not yet given, the needle fibres, but when Bawden came to Rothamsted and applied to tobacco mosaic the methods used with potato, he obtained the same needle crystals as Stanley's and most of his results were confirmed. Bawden & Pirie, however, carried the work a good deal further than Stanley and most of what I am about to say is based on their results.

There is no doubt that the material isolated from the juice of infected plants has most of the properties of tobacco mosaic. It is infective in concentrations of the order of one-millionth of a gram per c.c., increases very rapidly in the infected plant, and is transmissible in series from plant to plant indefinitely. The disease produced is identical in every respect with the disease of the plants from which the material was obtained in the first instance. With one important exception, with which I will deal in a moment or two, it has all the properties of the virus found

in naturally infected plants, such as resistance to chemicals, ageing, heat, enzymes, etc.

There is also no doubt that it is a protein. It gives all the usual reactions, and its analysis conforms to that usual in known proteins. Bawden & Pirie consider that it is a nucleoprotein but the American workers have not yet accepted this view, at least not in print. The discrepancy has a certain importance because of the implications and associations which a nucleo-protein nature suggests; but that is a technical matter which will no doubt soon be cleared up. Its composition is constant whatever the source of the material analysed and whatever the concentration of the substance it contains. It can be obtained from every host plant that the virus can actively infect. It is, therefore, not a substance peculiar to the tobacco plant or the tobacco-virus complex, but appears wherever the virus is able to multiply, even in hosts in no way related taxonomically to the tobacco plant, such as phlox or spinach.

It has not been found in any normal plant, even in very small amounts, and the methods of extraction are delicate enough to reveal it, even when it constitutes only a millionth part of the plant tissue examined. In the infected plant, on the other hand, it occurs in surprisingly large quantities. The juice of an infected plant contains from five to ten times as much soluble protein as that of a normal plant, and about 80 % of this soluble protein consists of the abnormal substance. From one to two grammes can be obtained from a litre of sap, the amount varying with the condition of the infected plants and the duration of their infection. Where all this excess protein comes from is unknown. It is conceivable that it is a modification of the protein already existing in the normal plant. Possibly the existing protein, perhaps the non-soluble portion, is converted into the new soluble form, and the cells, requiring the normal protein for their own purposes (and one must remember that the diseased plant, though damaged, is still a functioning organism, growing to a large size) are stimulated to replace the converted material by more of the original, which is in its turn converted, and so we get an accumulation of the new form, giving a total content of soluble protein much greater than is normally found. This theory would imply that the creative force, the synthetic or constructive power which converts the normal material into the abnormal, resides in or is a property of the abnormal substance itself. But there are other obvious possibilities. It may be, for example, that the *cell* produces the abnormal protein under the stimulus of the abnormality, a theory which evades the necessity of giving reproductive powers to the protein itself. The fact is that we have as yet no evidence

at all as to the mechanism or the source of this huge development of a foreign substance.

From solutions of the protein can readily be obtained needle-shaped bodies resembling crystals which are visible under the microscope. They average in length about two to three hundredths of a millimetre. They can easily be dissolved and again obtained, and the process can be repeated indefinitely. But as, I have said already, it has been shown by Bawden that these crystals are not necessarily pure, and contain a fraction which is not virus protein and can be removed by digestion with trypsin. This has been beautifully demonstrated, by means of the anaphylactic reaction, by Chesters, who showed the presence of the impurity in the American ammonium sulphate preparation and its absence in the purified material prepared by Bawden.

When a solution of the protein reaches a certain concentration it reveals a new property, becoming birefringent and showing anisotropy of flow. This property indicates that the constituent particles are rod-shaped. When the concentration is high, so that the rods cannot move about freely, but are necessarily arranged in parallel bundles for lack of space—like matches in a match box—the solution is permanently birefringent. When the concentration is of a lower order, the rods are able to move comparatively freely in all directions and there is no birefringence; but on the formation of currents or eddies in the liquid they assume the parallel arrangement in these areas, lying longways in the direction of the flow, with resultant anisotropy of flow. (This has been ingeniously shown by Bawden & Pirie, who put a goldfish in a solution of the protein, and the movements of its tail produced eddies which showed the birefringence.) The property is greatly affected by impurities, notably by breakdown products of the virus protein, and it is not possible at present to estimate the length of the rods with any precision though it seems that this must be at least ten times as great as the width. The width can be determined accurately from X-ray analysis, and is found to be 150 A. The cross-sectional area amounts to 20,000 A.^{2}, and is probably triangular, and these dimensions are constant for all concentrations of the protein.

The molecular weight and the particle size could be derived from the rate at which the particles come down in a centrifugal field, if the particles were spherical. Since they are rod-shaped it is scarcely possible to arrive at a really reliable value for the particle size. The cross-section shows that the molecular weight is large, and if we take the length as ten times the width and the specific gravity as 1·37, the minimum molecular weight

must be of the order of 20 millions. This is enormous. It is larger than the largest haemocyanins, and is approached only by one known substance, a thyroglobulin polymer which is estimated to have a weight of 15 millions. The new protein is at least a very unusual substance, unlike anything found in the sap of normal plants.

There is reason to believe that these rods are not the ultimate constituents, but are aggregates of subunits arranged in linear form. This is indicated by the fact that in the purified state the protein has lost the outstanding character of a virus, namely filterability. Every operation which precipitates the protein such as simple high-speed centrifugalization, precipitation by alcohol, by acid or by ammonium sulphate, entails this loss of filterability. We know that, in untreated sap, the virus can be filtered through membranes of a pore size of 53 mμ., but when it is purified it will no longer pass a membrane with 450 mμ. pores. It looks as if in the juice and, probably, in the living plant the virus exists in a smaller, more discrete form, but in the isolated state it has undergone an aggregation or polymerization—which must be linear, because the width remains the same. The existence of such subunits within the particle, of a length of about 20 A., is shown by the X-rays. There is other evidence that in the crude sap the protein is present in a different molecular arrangement —for instance, the absence of anisotropy in the sap, although it is a solution of 0·2 %, and the smaller infectivity of the isolated virus whose serological titre, nevertheless, remains the same as that of the virus in the sap. Up to the present it has proved impossible to disaggregate the protein again by any method tried.

Proteins of this type have now been isolated by Bawden & Pirie from three strains of tobacco mosaic and two strains of cucumber mosaic having a serological affinity to tobacco mosaic. The diseases are clinically distinct, and the corresponding proteins are also characteristically distinct. The protein varies as the virus varies. From other diseases, such as certain of the potato mosaics, these workers have obtained infective nucleoprotein precipitates which are amorphous and do not give the needle crystals, and which have a different composition on analysis. These proteins are susceptible to trypsin and so cannot be purified by its means, but they can be obtained by high speed centrifuging. Very interesting, too, is their recent work on bushy stunt of the tomato since, from that disease, they have isolated infective proteins which are truly crystalline. They are regular in all their dimensions, and therefore, of course, do not show double refraction; but they are infective in very low concentration, and in this case also there is no reason to doubt that

they are the virus. Other viruses examined in America have yielded similar proteins, so we have good reason to believe that a protein constitution of virus is not a unique phenomenon found only in tobacco mosaic.

There remains now to consider the important question—is this protein actually the real virus? If we could be quite certain that the purified protein is really homogeneous, really pure, the question would not arise. So far as intensive study has shown, virus proteins have been prepared which do seem to be really homogeneous. But it is always open to any one to assert that they are not, and that the virus is really present as an impurity in the protein preparation. Although the protein is present only when the virus is present, and is specific, it may yet be a reaction product produced by the plant in response to the virus, it may be a symptom just like any other symptom, and its infective properties be due to the presence in it of the true virus, from which it has not yet been separated. This assertion is one which cannot be directly disproved. One remembers the presence of heavy water in water thought to be pure and of argon in pure nitrogen. There is always a possibility that some test as yet unthought of, or some increased refinement of the existing tests, may reveal such impurity. The theoretical possibility must always remain, but the mass of evidence against it is now so large that we are entitled to disregard it, and the onus of proving its existence is transferred to those who assert it.

Certainly there can be no large impurity or inhomogeneity. The constancy of the product obtained from the most varied sources is enough to show that much; and also the infectivity which is regularly demonstrable in a concentration of 10^{-8} to 10^{-10}. Neither test is very refined, but nothing has been found that would suggest the presence of an impurity at all. Any procedure that removes protein lowers the infectivity, and the activity declines *pari passu* with degradation of the protein. The temperature or the degree of acidity or alkalinity which destroys the protein also destroys the activity, and it has not been found possible in any way to dissociate the protein from the virus. The hypothetical contaminant must have the same isoelectric point. It must also have the same molecular weight, since in the analytical centrifuge the pure protein gives the sharp sedimenting boundary of a pure molecular species. In short the contaminant must have the same physical properties as the protein and it is gratuitous to postulate the presence of two substances where one is enough to satisfy the data.

It would seem, then, that in the case of tobacco mosaic at least the old dispute between the vitalists and non-vitalists had been resolved in favour of the non-vitalist hypothesis. For, certainly, whatever exactly

the vitalists do mean, they would not consider that a pure chemical substance possesses all the properties they assign to organisms. It has, however, been maintained that all the work I have been describing to you does not exclude the possibility of the virus still being an organism. All that has been shown, says this argument, is that the isolated material has the property of linear aggregation, possibly even of true crystalline aggregation, under certain conditions of stress, such as high centrifugal force or precipitation, and that it gives the chemical reactions of a protein. But it has not been shown that these aggregating units are not themselves organisms. The isolated material, it is granted, is the virus, but why should not the ultimate units still be organisms and function as such in the disaggregated condition which they have when actually in the plant? Here again we come up against the question of homogeneity and, to some extent, against terminology. But there are positive objections to this argument. No known organism consists exclusively of protein. So far as we know at present, every recognized organism contains diffusible constituents, which can be leached out by appropriate means; but the virus proteins contain no diffusible constituents of any kind. Again, in every known organism, water is an integral part of its make-up, bound up with it and united to it in an intimate association. But that is not the case with the virus proteins. It does not unite with water. Water may penetrate between the particles, and does so to an extent which varies with the concentration of the solution. But it is a purely external relationship. The actual particles remain the same, as is shown by X-ray analysis in all concentrations of the material. Further, it has been shown by Bernal, again by the X-ray, that within these actual particles the structure is regular, and these regularities persist at all concentrations. The scale of this regularity is of the order of 20 Angstroms, very small therefore, smaller than any particle hitherto observed that may be claimed to be a living organism, and in character they are intermediate between a denatured and an undenatured protein. If there were any more complex material there, any vital constituent within the particle, it could only be a quite small fraction of the bulk of that particle. There does not seem to be any ground for the view that the constituent particles are organisms in any ordinary meaning of the word.

We are driven, then, to the conclusion that in the case of tobacco mosaic and its allies the actual virus is a protein, a chemical substance. But that is not to say that all viruses are proteins and nothing else. We know already that even within the tobacco mosaic group the infective proteins differ among themselves and have characteristic properties

which distinguish them from one another. We know, too, that while such a virus as the potato virus yields an infective protein, of liquid crystal type, it has not yet given anything but amorphous precipitates on isolation, and on analysis it is different from the tobacco mosaic protein. And, as I have said, Bawden & Pirie have recently isolated from the bushy stunt disease of tomato a protein which is fully crystalline, showing no double refractive flow, and with yet another analytical composition. It does not require much imagination to conceive that there may be a scale of increasing complexity of composition among the viruses, and that as one goes up the scale one may eventually cross the shadowy boundary between what may be called living and what must be called non-living. There may in fact be viruses which are truly organisms. But it does seem to be established that there are some viruses which are not.

AN INVESTIGATION INTO THE "STRIPE" DISEASE OF NARCISSUS

I. THE NATURE AND SIGNIFICANCE OF THE HISTOLOGICAL MODIFICATIONS FOLLOWING INFECTION

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(With Plates VI and VII and 2 Text-figures)

Introduction: Symptoms of the disease

During the past few years it has become increasingly evident that the disease known as "Stripe" is spreading rapidly among commercial stocks of Narcissus in this country. Nevertheless, our knowledge of the disease has hardly advanced since 1932 when Hodson (1932) pointed out that singularly little accurate information existed regarding the trouble. Although there is usually little difficulty in recognizing "Stripe", the symptoms displayed by affected plants vary so widely that some doubt exists as to whether it is a single disease or whether the name, as used at present, is really being applied to several different diseases which have not yet been recognized as separate entities, and which may later be shown to be caused by different pathogens.

The one feature common to all cases so far observed is a discoloration of the foliage and flower stalks due to abnormal distribution of chlorophyll in the tissues. Even this symptom, however, does not present a uniform appearance throughout the group, both the colour and shape of the affected areas varying, though they are usually constant within a variety. Two main types of discoloration have been observed. In one, the colour ranges from a green only slightly yellower than the normal to a bright yellow, while in the other it is a silver grey. The yellow-green type may take the form of longitudinal stripes of varying length and width or of mottling, the affected areas being rounded in outline and frequently covering a large proportion of the leaf surface of the affected plant. The silver grey type is present as longitudinal stripes extending practically the entire length of the leaf. The flower colour is also broken by light streaks or patches in some varieties.

In addition to one or other of these forms of discoloration other symptoms are frequently present. In some varieties the surface of the leaves becomes roughened owing to the formation of longitudinal corrugations of varying length. This condition is seen in an extreme form in the variety Czarina where ridges of tissue stand out prominently from the surface. The roughening usually occurs in discoloured areas, but the surrounding tissue is sometimes quite normal though the ridges themselves are yellow-green in colour. A somewhat similar condition may be found after bulbs have been subjected to hot-water treatment for eelworm, but from our observations it would appear that the ridges of tissue so produced are not discoloured in the manner typical of those associated with "Stripe", and there is, further, a tendency to cork formation which is not apparent in comparable symptoms in the diseased condition. The occurrence of the ridges on the surface of diseased plants is more widespread than might be supposed from superficial observation, for while they are obvious in some varieties, such as Czarina, they are detected only by very close observation in others in which they remain small and inconspicuous.

An entirely different symptom is found among infected plants of certain "Trumpet" varieties, notably King Alfred, the plant being severely distorted and commonly bent through a wide angle. In these cases the discoloured areas are often bright yellow, especially on the inner curve of the bend, the outer curve of which is frequently normal in appearance. Minor distortions of flower stalks and leaves are not uncommon, leaves quite often turning through a right angle in the plane of the surface.

The symptoms described in the preceding paragraphs are those encountered by us during the past growing season, either on the bulb farms visited or in our own experimental gardens and houses. There may well be others which we have not so far observed, for, as we shall show later, the origin of these symptoms is such as to allow of wide variation in the appearance of affected plants.

Histological modifications corresponding to different types of symptoms

As a first step towards a better understanding of these apparently dissimilar symptoms and of the disease, an investigation was made into the modifications of internal structure resulting from infection. Work on "Stripe" is severely handicapped at the present time by the lack of any technique for artificial transmission of the disease. McWhorter (1932) claims to have transmitted the apparently similar "Grey disease" in

America, but the inoculation methods tried in this country have not so far proved successful with "Stripe". The scope of this investigation is therefore restricted to an examination of plants showing various types of symptoms collected from bulb fields and our own gardens.

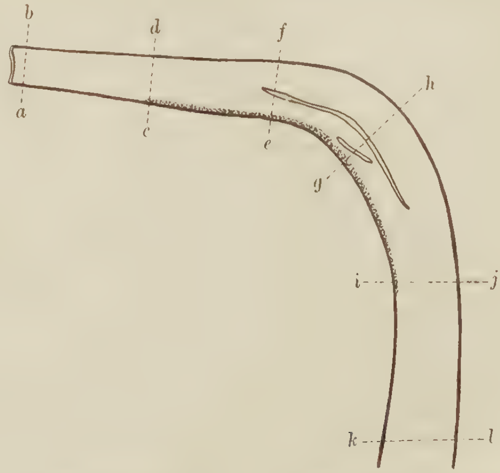
The ridges of tissue produced in the leaves of Czarina were first examined, and by cutting sections of a large number of leaves showing different stages of development the origin and mode of growth were determined. The palisade tissue in healthy leaves is represented by a single layer of elongated cells varying little in length except towards the edges of the leaf where they are more nearly isodiametric. A cross-section of a leaf from a diseased plant shows occasional cells in this layer to be devoid of chlorophyll and slightly larger than the neighbouring cells. The epidermal cells bounding such parts of the palisade are usually larger than normal. This is the first stage in the development of the ridges. The affected palisade cells continue to enlarge especially in the radial direction, where the spongy mesophyll offers less resistance than the palisade tissue which is more closely packed in the tangential plane. At the same time pressure exerted on the epidermis tends to push apart the cells in spite of their increased size. Eventually the enlarged palisade cells divide, a cross-wall being formed in the tangential plane. The products of division lengthen and, by this time, the tips of the cells protrude through a gap in the epidermis where two adjacent cells have been pushed apart. The width of the abnormal tissue as seen in cross-section depends upon how many adjacent palisade cells in the transverse direction are affected; usually two or three enlarge simultaneously at the beginning. In the longitudinal direction rows of cells of considerable length behave similarly, thus forming the ridge instead of a rounded projection. Further division and elongation of the newly formed cells result in the production of a tissue of appreciable size, replacing the palisade and projecting beyond the surface of the leaf. It has been noticed that the form of the cells in these proliferations varies even on the same leaf. In some they are long and narrow while in others they are short and practically isodiametric. This difference depends entirely upon the relation between the rate of elongation and the frequency of division. In some cases division of the palisade cells takes place before any appreciable elongation has occurred (see Pl. VI, fig. 3), and the daughter cells also divide before they have become much larger, thus producing a small-celled tissue as opposed to the large, radially elongated cells resulting when division is delayed until considerable increase in size has taken place. Intermediate conditions between these two extremes may also be seen, but whatever the final

form of the cells the origin of the proliferation can always be traced to the palisade. Pl. VI, figs. 1–3 illustrate the initial changes in the cells of this tissue which lead to the ultimate formation of the ridges in Czarina. The behaviour of the epidermis varies. Occasionally the rate of division and growth of the cells results in an increase in length of the epidermis which keeps pace with the production of tissue from the palisade, and in this case it remains unbroken, bounding the proliferation on the outside. When this happens it is usually found that the cells below are of the small isodiametric type denoting frequent divisions. Where the palisade cells elongate appreciably before division they push apart the epidermal cells at a comparatively early stage.

The origin of the ridges in Czarina having thus been established, a number of other varieties showing similar symptoms were examined. In every case the same developmental stages could be recognized. Among the varieties examined were Sir Watkin, Weardale Perfection, Minister Talma, Emperor and Golden Emperor. Attention was then turned to varieties in which no distinct ridges are apparent but in which the surface of the leaves becomes roughened locally, and it soon became evident that there was no essential difference between the origins of the roughened areas and the ridges in Czarina. The first specimen examined was a plant of Princeps, and the same proliferation of the palisade was observed. Fewer divisions take place however, so that less new tissue is produced. The ridges thus project but little beyond the surface, and as fewer adjacent rows of cells are involved they are also narrower than those found in Czarina. A number of such ridges running parallel and close together make the surface slightly rough to the touch although the corrugation is inconspicuous and not easily recognizable.

In a further investigation plants were selected in which the only noticeable symptom of the disease is discoloration. A good example of this type is afforded by one of the Triandrus hybrids. This variety shows extensive discoloration in the form of yellow-green mottling, but no ridges or roughening of the surface are apparent. We do not propose to discuss here the effect of the disease on chlorophyll or the origin of the different colours observed in affected regions, beyond stating that no chloroplasts have been seen in the cells of mature proliferations or in badly chlorosed areas. The significant fact for present purposes is that palisade cells in these plants show exactly the same tendencies as lead to the production of ridges in the varieties previously examined. Occasional cells are seen to be larger than their neighbours and devoid of chlorophyll. The epidermal cells opposite these sections of the palisade

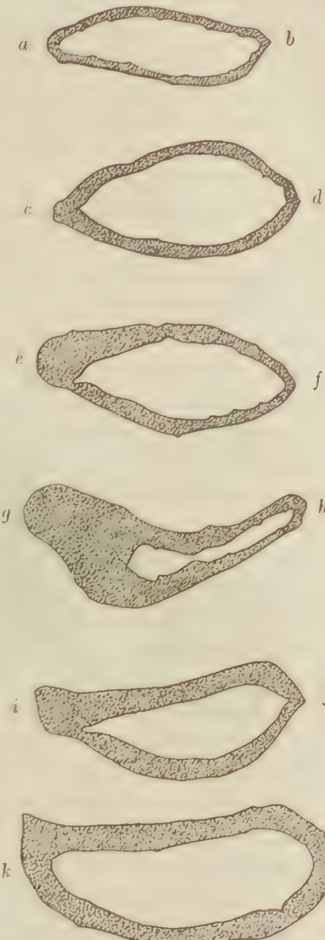
are often enlarged also. Less frequently a few divisions occur in the palisade mesophyll producing small ridges even less conspicuous than those in the roughened areas already described. Were they more closely grouped they might even so be detectable, but the affected rows of cells are rarely close together. Ridges are occasionally found in which division and enlargement have proceeded as far as in those causing roughening of the surface in other varieties, but being narrow and isolated they are not easily observed except by examining a section of the leaf. This is more particularly the case towards the end of the season, when the tendency to proliferation becomes more pronounced. Many plants of different varieties showing yellow-green discoloration were examined and all



Text-fig. 1. Distorted flower stalk in a "striped" specimen of Mon Tresor.

showed the same signs of incipient proliferation. This is true also of those in which the leaves showed silver grey striping, but in this type additional modifications are apparent. Not only is the palisade of the affected region lacking in chlorophyll but the spongy mesophyll is also chlorotic. The palisade cells divide without much enlargement, and there is a rather loose tissue of isodiametric colourless cells below the epidermis. The cells towards the surface of the leaf have thicker walls than those farther in. We have not yet seen any positive indication that the spongy mesophyll, besides having no chlorophyll, takes part in the proliferation, but it is difficult to rule out this possibility. The absence of chlorophyll from this tissue as well as from the palisade no doubt accounts to some extent for the distinctive colour of these areas.

The next plants to be examined were those showing severe distortion. A good example was found in a specimen of the variety Mon Tresor. The flower stalk curved through a complete right angle, the inner edge of the bend showing severe chlorosis and the outer edge being green and apparently normal. This appearance is typical of distortions of the type commonly known as the "King Alfred Stripe". The stalk was cut transversely at several points on the curve and above and below it, and Text-fig. 2 shows the form of the cross-section at the levels indicated by dotted lines in Text-fig. 1. At the level of the first cut (*a–b*) the hollow stalk consists of a ring of tissue of practically the same depth at all parts. The outermost tissue is an epidermis of the normal type with numerous stomata. Immediately below is a single layer of cells forming a continuous ring, well packed with chloroplasts and resembling the palisade layer in the leaves. Inside this the cells form short chains with wide air spaces between, making a tissue similar to the spongy mesophyll. The remaining tissue of the stalk consists of large thin-walled cells containing no chlorophyll. At a rather lower level (*c–d*) there is a slight increase in the depth of the ring of tissue at that part of the circumference corresponding to the inner edge of the curve. This increase is due to the production of a tissue of rather small isodiametric cells containing no chlorophyll and with slightly thickened walls. The epidermis remains intact, the growth keeping pace with the increase in circumference of the stalk. The new tissue lies immediately below it, and when this becomes



Text-fig. 2. Cross-sections of the flower-stalk illustrated in Text-fig. 1, at the levels indicated by the dotted lines.

extensive the differentiation between the outermost layer and the remainder of the cortex disappears. The whole area lacks chlorophyll, and there is gradual transition from small cells with thick walls beneath the epidermis to large thinner walled cells towards the centre. The latter show no change from medullary cells occupying a similar position at the higher level. A series of sections cut at different levels and including the region of transition from the normal structure to the abnormal thickened region afforded some indication of the origin of the new tissue. The first sign of any irregularity is the occurrence of cells containing no chlorophyll and slightly larger than the normal in the layer immediately below the epidermis. The elongation, though sufficient to be significant, does not proceed very far. The cells may occasionally double their length but never, so far as we have seen, exceed that. The tissue resembling the spongy mesophyll in leaves is devoid of chlorophyll but does not appear to take part in the proliferation. In the early stages it can be recognized by the characteristic arrangement of the cells, lying between the products of cell division and the medulla.

The next cut (e–f) revealed an increase in the extent of proliferation, and the cross-section at the middle of the curve (g–h) showed the maximum increase in thickness. Here the small-celled tissue has grown to a thickness many times that of the ring of tissue in the normal parts of the stalk and, furthermore, extends round an appreciable proportion of the circumference. The epidermal cells and those below, besides developing thick walls, show a tendency to form cork so that a tough compact tissue results having little elasticity. Subsequent sections at progressively lower levels show a similar sequence to those above the curve, and eventually the normal condition is again found in the straight part of the stalk. At the middle of the curve the normal structure is completely destroyed in the region where the proliferation occurs and longitudinal growth ceases. In other regions at the same level the structure is perfectly normal and there is abundant chlorophyll in the cortex. Hence with normal growth proceeding except in one sector of the circumference the stalk grows round the inextensible region forming a bend. As the proliferation is only of limited length, normal growth takes place both above and below the affected region. In the course of examining the stalk very much smaller proliferations were encountered having precisely the same mode of origin and development as the large one. These caused no distortion and produced ridges on the surface resembling those found in Czarina. The occurrence of distortion therefore appears to depend entirely upon the extent of proliferation, particularly in the tangential plane.

DISCUSSION

It is evident from the observations recorded above that the disease has three noticeable effects on the internal economy of the plant:

(1) Inhibition or destruction of chlorophyll causing discoloration of affected regions.

(2) A stimulus to cell division in the epidermal and palisade tissues of the leaf and in corresponding tissues of the flower stalk.

(3) A stimulus to growth of individual cells in the tissues mentioned in (2).

While each of these effects has been observed in all the varieties examined, the relative magnitudes are by no means constant and the visible symptoms vary accordingly. Since the appearance of a diseased plant depends upon a combination of three factors, each of which is capable of wide variation in degree, the number of possibilities is extremely large and is made even greater by the production of secondary effects such as distortion. The development of a particular effect in a plant depends upon the internal and external environments rather than uneven distribution of different casual agents. The influence of the internal environment is shown by the distinctive nature of the symptoms developed in each variety when several are growing together under similar conditions, while the power of the external environment is shown by the modification of the symptoms under changed conditions. Some plants of the variety Princeps growing in our experimental gardens were noted as being badly infected with "Stripe" at the beginning of 1936. The most obvious symptom was a well-marked chlorosis of the leaves, and towards the end of the season there was a slight roughening of the surface. During the summer some of the bulbs were lifted and planted in pots in a heated glasshouse. This season the plants in the house showed only very faint chlorosis but more marked roughening of the surface, while those in the gardens again showed severe discoloration (see Pl. VII, fig. 1). A similar result was obtained with plants of a Triandrus hybrid. A plot of this variety in our gardens showed severe yellow-green mottling on the leaves, and on 27 February two rows of bulbs were planted in pots and transferred to the heated glasshouse. The subsequent growth of the leaves of these plants showed such a diminution in the severity of the chlorosis as to appear practically clean, whereas the fresh growth of the leaves in the gardens showed mottling of the same intensity as before (see Pl. VII, fig. 2). With the advent of warmer weather, however, the same reduction of mottling became evident in these plants also, so that eventually leaves from both sets of plants pre-

sented a similar appearance. There was a severely mottled area at the tip and practically clean growth below. Since the leaves grow from the base the distance the mottling extends from the tip affords an indication of the relative periods of exposure of the plants to cold conditions, and a number of measurements gave a mean distance of 6·4 in. for plants grown throughout in the gardens and 4·5 in. for plants transferred to the glasshouse in February. The discoloration in the mottled areas becomes less marked after a time in the warmer conditions, and some plants which only appeared above the ground after the weather had become warmer appeared almost clean, suggesting that the disease inhibits the formation of chlorophyll rather than destroys it and the inhibition becomes less effective as temperature rises. The tendency to proliferation, on the other hand, becomes stronger under the warmer conditions. We have noticed more pronounced ridges on the leaves in the heated house than on the leaves of plants of the same variety growing outside, and many varieties produce detectable proliferations only in the warmer weather towards the end of the season when the discoloration may practically disappear.

The causal agent of "Stripe" is still obscure, but from our observations there would seem to be at least three components each having different optimal conditions for activity. Although all three are present in a diseased plant, the different behaviour of individual cells gives the impression that they can exist separately and are not uniformly distributed throughout the tissues affected by the disease (see Pl. VI, fig. 3). The symptom picture, the reaction to environment, and the observations recorded here are all suggestive of a virus complex, and although the disease has not yet been transmitted artificially we believe it will eventually be shown that such a complex is the cause of "Stripe". In this connexion it is of interest that during the examination of sections from diseased plants we have frequently noticed large bodies in the cells, lying close to the nucleus and resembling the *X*-bodies produced by some virus diseases. These bodies have not so far been seen in healthy plant material. Owing to the simple nature of such bodies and the lack of any characteristic feature by which they can be identified it cannot be stated that those observed during this investigation are necessarily the result of virus activity, but we have seen them only in diseased material although we have examined a large number of sections from healthy plants. They can be seen both in stained and unstained preparations but are most apparent after the fixing and staining techniques used by Sheffield (1931) for the examination of *X*-bodies. Pl. VII, figs. 3, 4 are photographs from sections treated in this way and show the bodies in question in the epidermal cells of infected leaves.

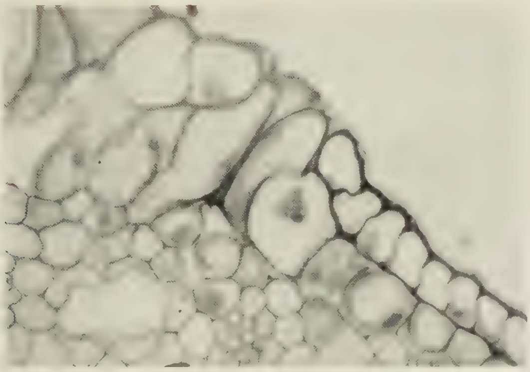


Fig. 1.

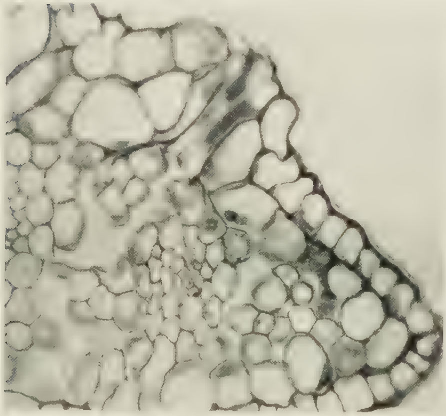


Fig. 2.

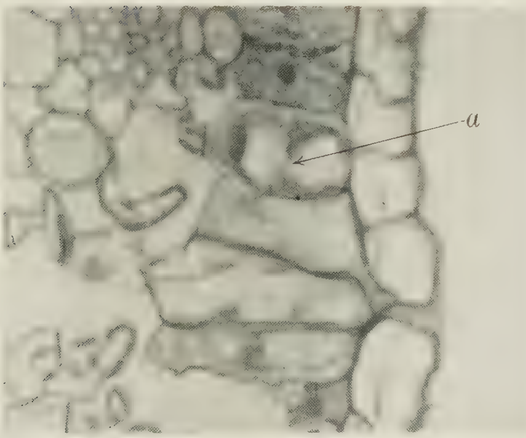


Fig. 3.

CALDWELL AND JAMES.—AN INVESTIGATION INTO THE "STRIPE" DISEASE OF NARCISSUS (pp. 244–253)

Fig. 1.

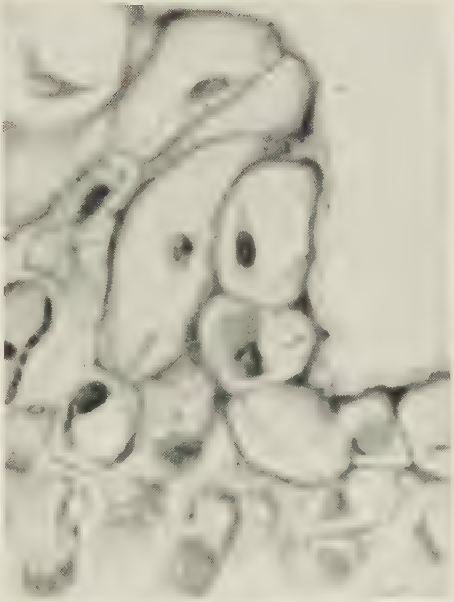


Fig. 3.

Fig. 2.

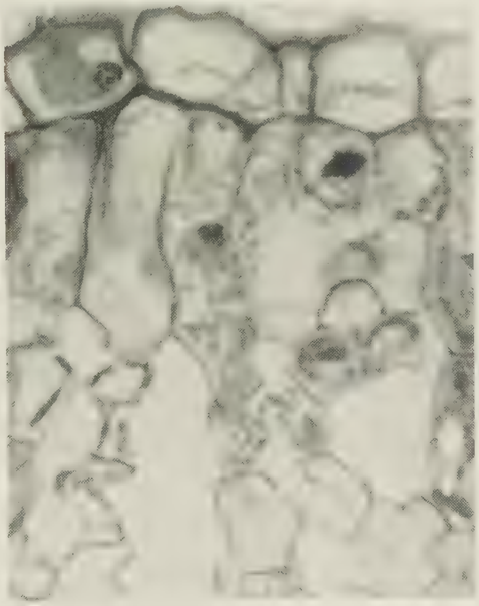


Fig. 4.

CALDWELL AND JAMES.—AN INVESTIGATION INTO THE "STRIPE" DISEASE OF NARCISSUS
(pp. 244–253)

SUMMARY

The wide variation in appearance of infected plants gives rise to the question whether the name "Stripe" is being applied to one or more diseases. The histological bases of the various types of symptoms are described, and it is shown that they are all produced by the same three factors acting with varying degrees of relative intensity.

It is suggested that the disease is caused by a virus complex having at least three components. Inclusion bodies in the cells of diseased plants are described which resemble the *X*-bodies associated with some virus diseases.

This investigation is being carried out under the auspices of the Agricultural Research Council.

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EXPLANATION OF PLATES VI AND VII

PLATE VI

Fig. 1. An early stage in the development of a ridge on the surface of a leaf from an infected plant of Czarina. Palisade and epidermal cells have enlarged but no division has yet occurred. The section is taken near the edge of the leaf where the palisade cells are more nearly isodiametric than they are in the central region. Note the inclusion body lying alongside the nucleus in one of the enlarged palisade cells.

Fig. 2. A rather later stage than that shown in Fig. 1. An enlarged palisade cell has divided forming the two cells on the left of the proliferation. A gap has been made in the epidermis through which elongated palisade cells are protruding.

Fig. 3. Transverse section of a leaf of Czarina showing elongation of palisade cells without division and (at "*a*") a cell which has divided after comparatively little enlargement.

PLATE VII

Fig. 1. Leaves of Princeps, (*a*) from the glasshouse, (*b*) from the gardens.

Fig. 2. Leaves of a Triandrus hybrid, (*a*) from the gardens, (*b*) from the glasshouse.

Figs. 3, 4. Sections of leaves from diseased plants showing the inclusion bodies in epidermal and palisade cells.

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THE ANTITHETIC VIRUS THEORY OF TULIP-BREAKING[1]

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(With Plates VIII and IX)

The word "antithetic" is here applied to two naturally associated viruses to connote differences in symptom expression which relate to some inherent antagonism between the two viruses. The antithetic virus theory of tulip-breaking states that typical breaks result from the presence within the plant of two viruses which, on a physiological basis, are not only distinct but are also antithetic. One of these is called Tulip Virus No. 1, or "colour-removing"; the other is called Tulip Virus No. II, or "colour-adding". The theory, originally formulated in 1931, required a four-year period for proof and demonstration. Its confirmation involved isolation of the viruses, determination of symptoms induced by each, and finally, the production with measured virus mixtures of typical broken tulips comparable to commercial Rembrandts. This paper is a record of these steps.

Terminology

It is proposed that the term "breaking" be accepted as the general name not only of the effects of tulip viruses on tulip flowers, but also as the general designation of the effects of viruses on other flowers where similar pigmentation disturbances are brought about. For example, the flower colour change in *Lathyrus odoratus* infected with mosaic is associated with virus activity. Breaking in tulips, according to popular usage, refers to removal, addition, or rearrangement of anthocyanin pigments in various areas of the epidermis of the flower. If the usage of the term is restricted to these specific occurrences, it should not be confused with "flecking", which results from thinning of the ground

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tissues into hyaline areas, a condition well shown in flowers of mosaic diseased narcissus.

In the present paper an attempt has been made to avoid additional terminology in describing broken-tulip patterns. The literature, both popular and technical, is laden with terms such as "clotted", "flamed", "feathered", "flag", "splashed", "full", "self", "dark", "white break", "red break", "Bizarres", and "Bybloemen". The antithetic virus theory excludes the need of most of these except for what might be termed cataloguing. The terms "full breaks" and "self-breaks" as used by workers at the John Innes Horticultural Institution seem especially apt, and are here given preference. These are interpreted as follows:

Self-break. The condition in which darkening of the flower is prominent. This may result from a local increase in the density of epidermal pigments or an addition of epidermal pigments. The ground colour may not be exposed. The pattern may be clotted, feathered, or evenly diffused.

Full break. The condition in which epidermal pigment is removed; the exposed ground colour then becomes the dominant colour of the flower.

Typical break or average break. The condition characteristic of commercial Rembrandts where the balance between epidermal pigments and exposed ground colour (white or yellow) produces a "flashy" or "flag" break. The usual colour of the flower is intensified in some areas, unchanged in other areas, and is removed in still others.

In the following tables and discussions, Virus I refers to colour-removing virus; Virus II to colour-adding virus. The symptoms or types of break induced are represented as follows: Symptom-type-1 refers to full break (as described above), Symptom-type-2 refers to average break, and Symptom-type-3 refers to self-break. It will be explained that the Symptom-type induced is a function both of the viruses present and of the tulip variety concerned.

HISTORY

It has been suggested that there are two or three kinds of tulip-breaking, each effected by a different virus. In 1929, Brierley,[1] while working with bulb viruses at the Oregon station, recorded "yellow mosaic" plants among Farncombe Sanders Rembrandts and made specific

[1] Brierley, P. Unpublished notes dealing chiefly with insect transmission studies, a part of which is summarized in the *U.S. Department of Agriculture Yearbook* for 1928.

inoculations with them. Before this, McKay & Warner (1933) had collected a broken purple tulip which evinced an exceptionally dark break and which was designated as "Marie Stewart mosaic", from an obsolete or local varietal name. He concluded: "In the experimental work completed at the time this review of the literature was undertaken, there was evidence of more than one, possibly three, types of mosaic disease in tulips." Hughes (1934) gives a favourable consideration to the antithetic virus theory, but slightly over-emphasizes the etiological significance of added colour patterns.

The antithetic virus explanation of tulip-breaking was proposed by McWhorter (1932) as a *theory* dependent on general observation of the following facts: first, the extreme variation shown in a population of approximately 2000 Farncombe Sanders Rembrandts used in natural spread tests in 1930–1; second, the record on inoculations made by Brierley in 1929, and repeated by McWhorter in 1930 and 1931, indicated that the type of break induced is directly related to the type of inoculum used; third, the fact that outlying shoots of large, typically infected Farncombe Sanders clumps often bear distinctly darker or lighter flowers than are borne by the inner portions of the clump. This third observation suggested that the self and full breaks and their accompanying leaf patterns might be accounted for by two agents (viruses) which move at unequal rates through clumps and hence induce different symptom expressions in different shoots of the same clump; and that the "kinds of mosaic" used in previous inoculations were merely natural segregates from average virus combinations brought about by the breaking up of clumps in the usual cultural practices.

To prove this hypothesis it was necessary to start with a large, typical full-break clump in which natural virus segregation was evidenced, encourage natural segregation by selective breaking up of the clump, and use the secondary clumps as sources of inocula. If the hypothesis was valid these secondary clumps should yield different viruses. After the viruses were thus isolated, it was necessary to show how they could be recombined for controlled synthesis of tulip breaks. This work is presented in the following tabulations and discussions in support of the antithetic virus theory. A similar method for the separation of plant viruses was first employed by McKinney (1931).

All of the flower records were made on field-grown plants, because under greenhouse conditions colour-removing symptoms are retarded, inhibition of chlorophyll is modified, and the symptoms as a whole are masked.

INOCULATION TECHNIQUE

The hypodermic needle method of inoculation is very successful with tulip viruses. A standard inoculum consists of juice expressed from tulip stems and leaves, filtered through cloth and diluted 1 to 10 or 1 to 20 with sterile distilled water. Inoculations are made into stems while the plants are in bloom or shortly thereafter by means of a 20-unit Luer syringe and small (No. 27) needle. The success of the method depends on the use of a small needle. Four or five units of inoculum per stem are sufficient to insure a high percentage of transfer. Sister-bulb checks are used in all important tests, so that each inoculated plant is checked by a non-inoculated sister plant derived from the same bulb clump. The details of this method, which is comparable to the tuber-unit system widely used in potato work, are given in a paper on iris mosaic by Brierley & McWhorter (1936). All of these records refer to field plantings left in place for 2 years. The first year the pairs, consisting of bulbs planted in sister-bulb sets of eight, sixteen, or twenty, are rogued to obviously healthy matched pairs; then one plant of each set is inoculated. The tabulations of inoculations here presented list the number inoculated and the number which survived the following year. The effectiveness of an inoculation is indicated by the number of clumps in which symptoms developed divided by the number of inoculated clumps which survived. The few survivals recorded in some cases resulted from the depredations of gophers (*Thomomys bulbiverus*) and moles (*Scapanus townsendi*).

ISOLATION OF VIRUS I OR COLOUR-REMOVING VIRUS

It is well known that some commercial Rembrandts tend to produce flowers of reasonably constant pattern; others tend to show great individual variation. Noticeable variations in the chlorophyll distribution in the leaves may accompany the flower differences. Farncombe Sanders Rembrandt is an example of a named variety which varies greatly in appearance and development. Fortunately there was available for study at the Oregon station in 1930 a stock of over 2000 of these Rembrandts, among which two chief groups or segregations were clearly discernible. One group was composed of weak plants with strongly striped, sometimes yellow striped, leaves and much whitened flower; the other was composed of plants with mottled leaves and average full-break flowers. The striped condition, favoured as it had been in this case by the deliberate saving of weak plants, represented a natural segregation of the Virus I, which, as will be shown later, is dominant. Selected plants

among these weaklings were the sources of inocula for further Virus I segregation. Continued selection of these weak Rembrandts will obtain an almost pure Virus I; the term "almost" is used because it is probable that a pure type I virus cannot be maintained in tulip-bulb clones.

The following is a typical case history of selective segregation of Virus I and Symptom-type-1 from Farncombe Sanders Rembrandt. In 1930 inoculum from a striped leaf plant was injected into 103 healthy Farncombe Sanders by the standard hypodermic-needle method. Two months later, the inoculated clumps were dug, broken up into units of four to seven bulbs each and replanted—thus encouraging further segregation. Eighty-six of the 103 inoculations were positive (1931), and considerable variation appeared within each unit; these furnished abundant material for further selection. A strongly striped plant with almost white flowers was selected from these and used as a second source plant in 1931. This time, ten Clara Butt plants were injected with inocula from the white of the flower and ten similar plants with inocula from the leaf. In both cases infection was 100 %, but the leaf inoculum was noticeably milder in effect than that from the flower. The plant descendants from those inoculated with the white part of the flower were all dead by 15 April 1932, the time when they should have been coming into bloom, while a few of the plants inoculated from the leaf source produced small, misshapen, white flowers. All of our plants containing theoretically pure Virus I have died out during the season they exhibited symptoms.

Isolation of Virus II or colour-adding virus

Selective segregation and isolation of the colour-adding Virus II has proved more difficult than with colour-removing Virus I. Natural Symptom-type-3 segregates are comparatively rare, and even where a large population of diseased tulips has been available we have never found one that was entirely pure for this condition. Fairly pure segregations have been obtained in several instances, but the one which now (1936) seems to contain no trace of colour-removing virus has the following history. In 1930, inoculum from a naturally infected Vitellina exhibiting a typical break with mottled leaves was inoculated into nineteen King Harold and fifteen Bartigon. The transfer was 18/19 and 14/15, respectively. All of the King Harold positives were typical breaks for this variety, which *always* selfs. Twelve of the fourteen positive Bartigon were average breaks; the other two were entirely different, one tending towards a Symptom-type-1, the other being an almost perfect Symptom-type-3, with dark flower and unmottled leaves. In 1931

separate inoculations were made into Clara Butt from the dark flower of this plant and from its leaves. The flower inoculum gave (1932) a transfer of 9/10, all of which were perfect examples of Symptom-type-3. The leaf inoculum gave a transfer of 9/10, of which eight approached type-3, but the remaining one was a type-2 with mottled leaves, showing that in this case the Virus II had travelled faster and farther into the flower of the inoculum plant. In 1932 this selection of dark flower inoculum was repeated, this time inoculating Clara Butt and Professor Rauwenhof. The results were all type-3. This source has been maintained, and now in colour-removable tulips induces dark flowers which bear no trace of colour removal. The Virus II in the pure form does little damage to tulip plants and is therefore easily maintained in tulip clones.

The foregoing are specific case histories of tulip virus separation by selective inoculation. At the Oregon station a collection of what might be termed a "garden of segregates", derived from several varieties of tulips, has been maintained. It has been repeatedly shown that inoculations from different parts of a large clump which exhibits different symptoms will have different effects, indicating varying proportions of the viruses. Pl. VIII is an illustration of a case where obvious segregation has occurred.

SYMPTOMS PRODUCED BY THE VIRUSES

Table I, presenting the comparative effects of the two viruses, is based on several hundred inoculations made in the course of our investigations. This table must not be considered a description of tulip-breaking as it normally occurs. It depicts specific virus actions. The average broken tulip exhibits symptoms intermediate between these extremes. Tulip varieties representing all colours available have been used. In general, Virus I is a colour-removing virus, inhibits chlorophyll formation, greatly restricts growth, and is directly responsible for the recognition of tulip-breaking as a disease. Virus II or colour-adding virus has no effect on the ground tissue of the flower or on the ground colour, stimulates epidermal pigmentation, has no visible effect on the leaves, and has little effect on growth. Pl. IX shows the characteristic colour changes induced by these viruses in the pink flowers of the variety Clara Butt.

We have completed a property study of these two viruses but have not been able to separate them on a property basis. That they are really different is abundantly shown in their induced plant reactions. The subject of properties is reserved for a comprehensive paper on this subject, but the thought is here introduced that two viruses can be different,

antithetic, and even antagonistic—yet exhibit the same "physical" properties.

Table I

Symptoms induced in tulips by tulip Viruses I and II

Effect on	Colour-removing tulip Virus I	Colour-adding tulip Virus II
Plant size. All colours	Size of plant reduced 30–75%. Bulbs die out the second year	Size of plant reduced 10%
Leaves. All flower colours	Strong yellow-green stripe, X-bodies and virus type cell disintegration frequent. Leaves soon redden	No visible effect. No X-bodies
Stems	Purple pigment on stems reduced and rearranged	Epidermal purple pigments may become more conspicuous
Flowers, whites	Some clearing due to inhibition of ground tissue formation in areas which appear as hyaline spots referred to as "flecks"	Most white flowers turn pink or even strong red
Flowers, yellows	Do.	Some yellows turn red; others remain unchanged in appearance
Flowers, pinks	Turn white with only a trace of pink left. Pink remnants are darker than the original pink	All pinks tested turn a strong red. A trace of white is usually evidenced near the base. The colour "clots"
Flowers, purple shades	Some turn white with purple and dark red remnants; others white and darker purple	Turn very dark and beautiful purple. The colour "clots"
Flowers, reds	Most reds turn white with only a trace of red left; others darken (see Table III)	Turn very dark, almost black
Flowers, "blacks"	Darken and appear burned	Turn even blacker except on petal edges where white flecks appear

The relationship between tulip Virus I and tulip Virus II does not seem comparable to that between strains of a virus where the infection of a plant by one strain of a virus will protect the plant against infection by a related strain of the same virus. This infection interference is considered by Kunkel (1934) as an immunological reaction in the plant and thereby a measure of virus relationships; viruses which show infection interference are considered strains of the same virus, or at least of closely related viruses. In the course of these studies, Symptom-type-3 and type-2 plants have been successfully inoculated with type-I virus, so that the inoculates were completely changed. A type-II virus transfer into Symptom-type-1 has never been attempted, since type-I virus is known to be dominant. Virus terminology is as yet too unsettled to warrant a specific classification of the tulip viruses. They are different, but whether the difference is comparable to "species" or "strains" is a matter of

opinion. There is no evidence of immunization other than the *dominance* of Virus I; this would indicate, therefore, that the difference between these viruses is of a higher order than "strains".

SYNTHESIS OF NORMAL BREAKS

As stated above, two viruses were obtained through selective segregation from originally typical breaks. The separation of these viruses proved that typically broken tulips contain two viruses; it did not prove that such breaks could be induced without the presence of a third virus or factor. Proof that the two viruses in certain combinations are able to induce normal breaks was accomplished in two ways: (*a*) by injecting healthy tulip plants coincidentally with viruses from type-1 and type-3 plants; (*b*) by injecting healthy tulips with measured mixtures of the viruses in known proportions.

The symptoms induced by the two viruses, as recorded above, favour our interpretation that they are antithetic. Examination of the results from using measured mixtures of virus-bearing juices as inocula have yielded in terms of growth and colour responses, specific proof that these viruses are physiologically antithetic (see Table II).

Our first attempt at synthesis was made in 1931, using the fairly pure segregations available at that time. The virus-bearing juices diluted 1 : 10 were used as follows:

Virus I into nine Clara Butt induced 3/9, all Symptom-type-1.

Virus II into ten Clara Butt induced 4/9, all Symptom-type-3.

One part Virus I plus one part Virus II into nine Clara Butt induced 8/9, all Symptom-type-1.

One part Virus I plus three parts Virus II into nine Clara Butt induced 4/9, all Symptom-type-1.

Three parts Virus I plus one part Virus II into twelve Clara Butt induced 11/12, all Symptom-type-1.

The results of the test were disappointing because the type-1 virus proved completely dominant. At the same time these mixed virus inoculations were made, the 1 to 10 dilutions of each were injected *separately* into the same nine Clara Butt plants. This double inoculation induced 7/9 transfer of which five were Symptom-type-1 and two were Symptom-type-3 in character. The interpretation of these preliminary tests is that Virus I is dominant and that separate (coincident) inoculations may result in separate action of the viruses. Normal breaking was not duplicated.

Table II

Normal-broken tulip synthesis with virus combinations 1932 *inoculations; readings* 1933

Proportion and dilution	Ratio of dosages of viruses	No. plants inoculated	No. plants survived	No. positive	Distribution of Symptom-types induced	Total length in cm. of flower stems over no. of stems	Av. length in cm. of stems
One part of 1/100 Virus I to one part of 1/100 Virus II—combination inoculum	1 : 1	8	8	8	Two perfect type-1, five mild type-1, one type-2. Colour removing dominant	259/13	20
One part of 1/100 Virus I to ten parts of 1/100 Virus II—combination inoculum	1 : 10	6	6	5	Two type-1 and three type-2. Normal breaks typical	175/8	21
One part of 1/1000 Virus I to one part of 1/100 Virus II—combination inoculum	1 : 10	8	8	8	Two type-1, five type-2 typical normal breaks, one type-3. This mixture produced mostly normal breaks	634/17	37
One part of 1/1000 Virus I to ten parts of 1/100 Virus II—combination inoculum	1 : 100	7	7	5	No type-1, three type-2, two type-3	217/6	36
One part of 1/10,000 Virus I to one part of 1/1000 Virus II—combination inoculum	1 : 10	13	13	0	Inoculation failed. The transfer should have been at least 50 %. No explanation	—	—
One part of 1/10,000 Virus I to one part of 1/100 Virus II—combination inoculum	1 : 100	7	7	4	Virus II entirely dominant. Leaf mottle absent. There were nine flowers; only four showed a trace of colour removal. Symptom-type-3	222/7	32
One part of 1/10,000 Virus I to one part of 1/10 Virus II—combination inoculum	1 : 1000	7	7	5	All type-3	208/6	35
Check—1/100 Virus I only	—	5	5	5	All type-1, plants were killed down. No flowers produced	—	0·0
Check—1/100 Virus II only	—	8	8	8	All good type-3, all flowers selfed	—	—
1/100 Virus I and 1/100 Virus II separate inoculations	1 : 1	6	6	6	Five type-1, one type-2. Type-1 dominant	—	—
1/10 Virus I and 1/100 Virus II separate inoculations	10 : 1	7	7	7	Two type-1, four type-2 with type-1 tendency. One type-2 dark break	—	—
1/100 Virus I and 1/10 Virus II separate inoculations	1 : 10	12	11	10	Three type-1, six type-2 and one type-3		

Total length of the flower stems of all the type-1 inoculates 353/17. Average 20·7 cm.
,, ,, ,, 2 ,, 805/24 ,, 33·5 ,,
,, ,, ,, 3 ,, 526/15 ,, 35·0 ,,

The total length of flower stems of the comparable plants that remained healthy 1662/39. Average 42·6 cm.

Note. The plants inoculated with type-1 alone produced no flowers and hence are not listed in the above table of lengths of stems; the length was 0.

Using the excellent virus segregates available in 1932, a comprehensive series of combination inoculations was made. These were tabulated in Table II. One source plant was used for each virus. The coincident inoculations were made shortly after extracting the virus-bearing plant juices. The proportioned mixtures were allowed to stand several hours before inoculating so that possible interaction between the viruses might be initiated before they had a chance to begin development within plant tissues to which they were subsequently exposed during infection processes. Previous experiments had shown that the infectiousness of these viruses when inoculated separately is not materially affected by ageing within a period of 2 days.

The distribution of types induced by combination inoculations of different proportions of the viruses must not be taken too literally because even when pre-mixed inocula are injected there is always a chance for selective infection. The results, however, are considered indicative of the proportion of the two viruses necessary for typical tulip-breaking. The exact virus proportions constituting the physiological balance of the typical break (type-2) cannot be exactly stated until these viruses can be isolated and quantitatively measured. In these experiments typical breaks were synthesized from double-virus inoculations. If the proportions of diluted virus-bearing juices inoculated are true criteria, then a typical break may be considered an expression of tulip Viruses I and II where the concentration of the colour-adding II is at least 10 times that of the colour-removing I. This conclusion is based on the pink tulip, Clara Butt. The proportions of Viruses I and II required for the production of typical breaks in other varieties have not been determined, but it is probably the same since inoculation with juice from typical type-2 Clara Butt plants induces typical type-2 breaks in other varieties.

The dominance of the colour-removing Virus I seems proved not only by the specific inoculations here recorded but by the tendency of plants with a preponderance of this virus to segregate out during the propagation of commercial Rembrandts.

The strongest proof of the antithetic intervirus reaction which plays a role in the synthesis and stabilization of the average tulip break is the relative growth performance of plants which have received individual inocula compared with those which have received mixed inocula. In every variety of broken tulips that we have studied the type-2 plants are invariably larger than the type-1 segregates (or inoculates). The presence of the Virus II where Symptom-type-2 is present reduces the shock of Virus I and limits its ability to inhibit growth. This effect is

reflected in the height data of Table II, but the effects of selective infection, when combined inocula are employed, cannot be circumvented. The "length of stem" data are measurements to the base of the flower. The comparable height of the five plants which received Virus I must be recorded as zero, since the plants were dead by blooming time and produced no flowers. It is significant that all the mixed virus inoculations, including those mixtures consisting of equal proportions, produced flowers, even in cases where flower and leaf types showed that the Virus I had remained completely dominant. There is good agreement between long stems and the presence of Virus II except in the height class 37 cm. In this case where Viruses I and II were present in dilutions of 1 : 1000 and 1 : 100, respectively (ratio of I to II is 1 : 10), the departure from the expected stem shortening due to Virus I must be attributed to experimental uncertainty at this dilution. Since these experiments involve a 2-year planting and the records are taken during the second year, the extreme growth variations, which might have resulted from differences in sizes of bulbs planted, are largely eliminated, thereby making the stem length differences attributed to virus action relatively more significant.

The experiments of 1932–3 proved our contention that normal tulip-breaking, as exemplified in pink varieties, results from the interaction of two viruses. In the course of a study of properties which has just been completed, we hoped to obtain these viruses in purer form and repeat these synthesis tests. This quest was unsuccessful. We have been able to separate them only by means of the segregation method described above.

The behaviour of red-flowered tulips

A confusing point relating to tulip virus analysis is the fact that some dark red tulip varieties never break white or form the pattern which Hughes (1934) calls "full break". In these red varieties so far tested the factor for increased redness is fixed within the plant so that the presence of either virus is indicated by added colour; the plant, not the virus, controls the type of break. These red effects, for which some hidden factor within the plant is responsible, must not be confused with the reddening brought about by the colour-adding Virus II alone. In these unorthodox red varieties Virus I induces characteristic leaf mottle, but Virus II brings about no recognizable effects in the leaves. The type or types of virus present in these reds can be surmised by examination of the leaves and flowers, and proved by inoculation into pink varieties.

The similarity of physical properties of two viruses so different in physiological effect suggested that the factor for colour addition might

not be a virus but a plant-borne factor related to that which causes some red tulips never to break white or assume the full-break pattern. Cross inoculations were made from virus infected, non-red and red tulips into healthy red varieties with the purpose of finding some recognizable plant character which could be correlated with what may be termed the ability of most red tulips to break into white and red patterns, that is, to assume the full break condition. A selected list of these inoculations is given in Table III. In every case the inoculum used should have induced a full break had not some of the reds (those listed as selfed) contained a factor limiting breaking. No recognizable plant characteristic has been found capable of serving as an indicator of the type of break induced.

Table III

Tabulation of inoculations on red tulips. Inoculated plant record

Virus source variety	Symptom type	Variety inoculated	Year	No. plants inoculated	No. survived	No. positive	Type of break
Farncombe Sanders	2	Allard Pierson	1930–1	5	5	5	Self
Solfatare	2	Bartigon	1930–1	20	19	17	Full
Avis Kennicott	2	,,	1930–1	20	20	15	,,
Vitellina	2	,,	1930–1	20	16	14	,,
White Queen	2	,,	1930–1	20	19	16	,,
Clara Butt	2	,,	1932–3	7	7	7	,,
,,	1	,,	1932–3	8	4	4	,,
,,	2	City of Haarlem	1932–3	15	13	9	Self
Diana	2	Farncombe Sanders	1930–1	20	20	2	Full
Prince de Ligny	2	,,	1930–1	20	20	9	,,
Feu Brilliant	2	,,	1930–1	20	17	9	,,
Sieraad van Flora	2	,,	1930–1	20	16	6	,,
Bronze Queen	2	,,	1930–1	20	16	6	,,
La Tulipe Noire	2	,,	1930–1	20	18	3	,,
Professor Rauwenhof	2	,,	1930–1	20	13	13	,,
Farncombe Sanders	2	,,	1930–1	5	5	5	,,
Clara Butt	2	,,	1931–2	40	39	38	,,
,,	2	,,	1932–3	9	9	8	,,
,,	1	,,	1932–3	10	8	7	,,
,,	2	Feu Ardent	1932–3	16	9	8	,,
Farncombe Sanders	2	Feu Brilliant	1930–1	5	5	5	,,
Clara Butt	2	Harry Veitch	1932–3	12	10	8	Self
,,	2	Jacob Maris	1932–3	16	14	7	,,
Solfatare	2	King Harold	1930–1	20	19	17	,,
Avis Kennicott	2	,,	1930–1	20	16	15	,,
Vitellina	2	,,	1930–1	20	19	18	,,
White Queen	2	,,	1930–1	20	19	17	,,
Clara Butt	1	,,	1932–3	8	8	8	,,
,,	2	La Merveille	1932–3	8	8	8	Full
,,	2	Lucifer	1932–3	15	14	3	,,
,,	2	Prince Albert	1932–3	16	15	9	Self
,,	2	Prince of the Netherlands	1932–3	12	11	6	Full
,,	2	William Pitt	1932–3	16	15	14	,,
,,	1	,,	1932–3	8	7	6	,,
,,	1	La Tulipe Noire	1932–3	6	4	4	Self
Farn. Sanders Rembrandt	—	,,	1930–1	20	18	16	,,
Farncombe Sanders	2	,,	1930–1	5	5	5	,,

The red varieties so far tested which have proved incapable of full breaking are City of Haarlem, Allard Pierson, Harry Veitch, Jacob Maris, King Harold, Prince Albert, and the so-called black tulip, La Tulipe Noire. Allard Pierson and King Harold have been subjected to special study. We have injected both viruses separately and mixtures of Viruses I and II into these reds and then made return inoculations into pinks to determine whether the procedure would increase the factor for darkening. Passing the viruses through these special reds had no modifying effect on either virus. Moreover, in a comprehensive variety and colour test it was shown that no tulip variety tested, including several dark reds and La Tulipe Noire, had any direct effect on the inoculum.

The passage of a balanced virus mixture through these special reds had no effect on the mixture; the factor for inherent redness could not be picked up during subinoculation. A further check on this interpretation was made possible by inoculating Clara Butt with juice from healthy reds of the King Harold group. The flowers of the inoculates could not be distinguished from their sister-bulb checks.

The variety Allard Pierson[1] is an excellent example of a red tulip which does not show a trace of white even when inoculated with a purified Virus I. A test was made in 1935–6 to determine whether the juice of this tulip would behave like an antigen to the colour-removing virus. For this purpose juice from an average broken Clara Butt was used as inoculum representing a natural physiologically balanced mixture of Viruses I and II. The Clara Butt juice was filtered through cloth, cleared by allowing to stand and by decanting, then diluted with sterile distilled water as indicated below. The juice from healthy Allard Pierson was used undiluted. The mixtures of healthy juice and diluted virus-bearing juice were kept at a temperature of about 9° C. for 16 hr. before injecting them into healthy Clara Butt plants on 2 May 1935. In the tabulation below, "A.P." signifies healthy Allard Pierson juice and "C.B." refers to Clara Butt juice bearing an average virus mixture. The readings are for April 1936.

One c.c. C.B. diluted 1 : 20 plus 5 c.c. A.P. induced 11/15 average break
One c.c. C.B. diluted 1 : 100 plus 5 c.c. A.P. induced 10/12 average break
One c.c. C.B. diluted 1 : 200 plus 5 c.c. A.P. induced 9/13 average break
One c.c. C.B. diluted 1 : 1000 plus 5 c.c. A.P. induced 2/13 average break
Check C.B. juice diluted 1 : 50. Induced 11/12 average break
Check A.P. juice diluted 1 : 10. Induced 0/15 unbroken

[1] The writer has been informed on good authority that Allard Pierson listed as crimson maroon (Darwin) by Krelage in 1926 is a reselection of the old variety, Mrs Allard Pierson.

A thorough examination of individual flowers and leaves of this group of inoculates showed that the Allard Pierson juice had no modifying effect whatever. There was no significant variation in the degree of colour removal. It should be noted that in the highest dilution used, the virus-bearing juice was diluted 1 : 5000 when added to 5 c.c. of Allard Pierson juice, a proportion that would have given dark flowers were this factor which inhibits red pigment removal able to function as does Virus II, the colour-adding virus.

The conclusion from these observations on these special reds is that Virus II is entirely distinct from the factor for self-breaking in these dark red varieties. Moreover, the obscure factor for self break in these reds does not seem to be a virus and is not separable from the plants which contain it. It is certainly not the same as Virus II which enters into the average break and has the property attributes of a true plant virus.

INTERPRETATION OF TULIP-BREAKING INDUCED BY ANTITHETIC VIRUSES

The above data point to two viruses being present within the typically broken tulip plant. The viruses are the initial causal agents. The effect of the viruses is a function of proportion and of certain limiting factors within the plant. These limiting factors which regulate what may be termed the symptom display contingent to the viruses may be grouped as follows:

(*a*) The presence or absence of precursors capable of forming red pigments in the epidermis of white and yellow flowers.

(*b*) The presence or absence of substances capable of forming red pigments in flower epidermis which is normally purple.

(*c*) The presence or absence of factors which inhibit colour removal by Virus I.

(*d*) In some Triumph tulips a factor or factors are present which prevent viruses from changing the original colour pattern. In these cases the usual pattern merely shrinks in size, without yielding to differential colour changes.

The solution of these plant factors will lead to a biochemical analysis of formative anthocyanins and their determinants on the one hand, and to a genetical analysis of tulip varieties and groups on the other. Complete analysis portends difficult but fundamental research. At present we are initiating a more extensive study to determine the varietal range

of distribution of the factor, or factors, which inhibit the removal of red pigments.

The broken tulips known commercially according to the colour and group of their healthy prototype as Rembrandts, Bybloemen, Bizarre, etc., represent responses to physiologically balanced mixtures of two viruses. Many of these named varieties are of reasonably vigorous growth, though not quite equal to their prototypes, some of which are called "breeders" because of their vigour. The fact that these commercial broken tulips continue to grow and reproduce led to the old theories of "rectification" and the assertion that tulip-breaking is not a disease. The theory was that a broken clone became "rectified" with age. A further proof that such broken tulips represent a balanced virus condition was shown by deliberately overbalancing one of these established varieties by inoculating typical plants with Virus I. In this experiment, nine plants of the Bybloemen Violet Wodan were inoculated; five of these changed from the dark type of commerce to dominantly white flowered forms with only a remnant of purple. The average height of the flower stalks of the inoculates was 28 cm.; that of the check Bybloemen was 43 cm. This test is particularly significant since a 5-year record on this variety shows that it is one of the most stable of the broken Dutch breeders that we have kept under observation. It would seem, however, that the old term "rectification" may be justified technically as a descriptive term of the process through which newly infected tulips go while their virus burdens appear to adjust themselves into a physiological balance.[1]

In the present article, relatively little attention has been given to selfing versus clotting. These symptoms are inter-related between virus effects and plant factors. For example, those reds in which a full break cannot be induced tend to clot. Average mixtures of viruses tend to induce self-breaks, the term being used to refer to the condition known as "feathering". As the virus proportion approaches the pure II condition, clotting becomes more pronounced, and inoculates which receive the pure II virus exhibit clotting only. The appearance of the leaf is, however, a more reliable criterion of the dominant virus type than the pattern of the added colour.

With these viruses singled out and isolated in plant segregates and with an understanding of the limiting factors that are functions of tulip

[1] A further significance will be given to the term in a paper now in preparation which will prove that the probable ultimate source of the broken tulip is the group of bulb-perpetuated Chinese lilies.

varieties, one may proceed literally to make broken tulips to order. But even well-balanced, average-broken tulips are a menace to other tulips because of the danger of segregation and selective action of Virus I. Alone it is a killing virus. The Virus II, however, when pure deserves consideration as a possible tool for the creation of truly beautiful flowers. This virus adds to purples and pinks beautiful darker shades without materially reducing vigour. The patterns are always clotted and sometimes beautiful. This suggests the question: Can induced patterns be controlled?

As a result of observation of more than 5000 tulips broken with known or recorded inocula, the following general observations seem warranted. If a mixture of Virus I and II is taken from a plant showing a typical symptom complex (this connotes a balanced virus mixture) and inoculated into 100 tulips, 99 out of the 100 may be expected to show an average break and a similar pattern. The remaining plant may show selective segregation towards the type-1 or type-3 conditions. The tulips in the group will assume the same general pattern, but this pattern will usually not closely resemble that of the inoculum source plant. We have never been able to make a conspicuous pattern transfer even where inoculum and inoculates represented the same variety of tulip. The foregoing statements relate to physiologically balanced virus mixtures. When unbalanced virus mixtures are used, the induced colour patterns vary greatly in appearance. When a source plant containing a pure Virus I is used, there is usually no flower and hence no pattern. When a plant containing pure Virus II is used, there is better agreement between the patterns of the inoculum plant and the ones inoculated. The pattern of the flower colours induced remains uncontrollable.

SUMMARY

The term antithetic is suggested for viruses which are usually associated and which are physiologically antagonistic.

Tulip breaking results from the interaction of Tulip Virus I which inhibits flower and leaf colour and Tulip Virus II which adds flower colour but has no visible effect on chlorophyll distribution.

The established commercial broken tulips contain physiologically balanced mixtures of these two viruses.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES VIII AND IX

PLATE VIII

A Farncombe Sanders plant was inoculated with a tulip virus mixture in 1929. In 1930 it developed a typical break. Bulbs were then transplanted and the subsequent plants left undisturbed. In 1932, virus segregation occurred as indicated in this photograph.

PLATE IX

Fig. 1. A Clara Butt flower produced by a plant where tulip Virus I (colour removing) is present and dominant.

Fig. 2. A Clara Butt flower produced by a plant where both Virus I and Virus II are present and are "physiologically" balanced.

Fig. 3. A Clara Butt flower produced by a plant where tulip Virus II (colour adding) is present.

Fig. 4. Flower of healthy Clara Butt tulip.

(*Received* 11 *September* 1937)

McWHORTER.—The Antithetic Virus Theory of Tulip-breaking (pp. 254–270)

McWHORTER.—The Antithetic Virus Theory of Tulip-breaking (pp. 254–270)

THE RELATION BETWEEN POTATO BLIGHT AND TOMATO BLIGHT

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(With Plate X)

Epidemics of blight (*Phytophthora infestans* (Mont.) de Bary) on outdoor potato and tomato crops are frequent in Jersey. The early potatoes, variety International Kidney, are planted in January and February and blight usually appears in April; in May and June the crop is in full growth and disease may become prevalent in either month. The young tomato plants, varieties Sunrise and Devon Surprise, are transferred from boxes to the fields in May and June, and, except on rare occasions when the plants are attacked in the boxes, blight appears after the crop is well established in the open. In experiments where potatoes were interplanted with tomatoes in the field in spring, the former were almost killed with disease before the tomatoes were attacked. Under field conditions blight appears first on the potato crop each year.

Most growers assume that the disease passes from the potato to the tomato and, in order to prevent this, diseased potato crops in the neighbourhood of tomatoes are sometimes destroyed. The validity of this assumption has been tested in the present investigation. It may be noted that the symptoms of blight on outdoor tomatoes are similar to those of potato blight.

Experimental methods

Sources of inoculum. Pure cultures were not used. Fresh diseased tissue was taken and, if necessary, was kept in a moist chamber for 1 or 2 days to encourage sporulation. It was then floated on tap water to obtain a suspension of conidia which was used immediately for inoculation purposes. Diseased tissue was obtained from: (*a*) the early glasshouse potato crop in January, February or March, (*b*) the early outdoor potato crop in spring, (*c*) the late outdoor potato crop in summer and autumn, (*d*) the outdoor tomato crop in summer and autumn.

Inoculation experiments. Young potato plants (variety International Kidney) and tomato plants (varieties Sunrise and Devon Surprise) in pots were used, and also large detached leaves with the petioles in water.

The results obtained with detached leaves are not recorded since they were similar to those obtained with pot plants. Both varieties of tomato are susceptible to blight.

The suspension of conidia was applied to the lower surfaces of the leaves with an atomizer, and the plants were kept in a very moist chamber in the laboratory throughout the experiment. In all the trials, blight taken from either host was tested on both potatoes and tomatoes so as to compare the symptoms and to ascertain if the environmental conditions were suitable for infection. Severe infection was established in 5–7 days but in doubtful cases the experiments were continued for 10–14 days. Controls were sprayed with tap water from the atomizer before the latter was used for the spore suspension; the controls were placed in the same chamber as the inoculated plants.

Inoculations with blight from early glasshouse potatoes

These experiments were carried out in January, February or March, a period when no outdoor tomatoes are being grown. The spore suspensions were obtained from diseased potato leaves of the variety International Kidney. Inoculated potato plants or leaves always developed symptoms of ordinary potato blight, while inoculated tomato plants reacted as follows.

In 1935 two experiments were made, using blight from two distinct glasshouse nurseries. In one trial the plants remained healthy; in the other each plant developed a few spots of typical blight with abundant sporulation.

In 1936 the tomato plants were severely infected with potato blight 7 days after inoculation.

In 1937 two experiments were made, using blight from the same glasshouse but collected in January and February respectively. In all cases typical blight infection was established on inoculated tomato plants, but the number of lesions was small.

The results show that, in four out of five experiments, symptoms of ordinary blight developed on tomato plants inoculated with blight taken from glasshouse potatoes, in January, February and March.

Inoculations with blight from early outdoor potatoes

Spore suspensions were obtained from the very early outbreaks of blight discovered in April or May on the outdoor potato crop, variety International Kidney. At this time little, if any, tomato blight was likely to be present in the island. All inoculated potato plants became

severely infected in 5–7 days. On tomatoes the following results were obtained.

In 1933 six experiments were made, and of the thirty plants used twenty-nine remained perfectly healthy. One plant developed several small (no more than $\frac{1}{4}$ in diam.) black spots on the leaves; the spots did not enlarge and kill the foliage and no definite sporulation was observed.

In 1934 all six inoculated plants remained healthy.

In 1935 one plant remained sound but four developed numerous small black spots on the foliage similar to those of 1933 except that a little sporulation occurred. This was the first record of definite infection of tomato plants with blight from the early potato crop.

In 1937 two experiments were made and six plants remained free from infection, while four developed a total of seven spots similar to those observed in 1933 and 1935; no sporulation occurred.

The results show that, under the experimental conditions, blight from the potato infected the potato severely in 5–7 days, but often failed entirely to infect the tomato. Where infection of the tomato was established, the symptoms produced were quite unlike those found on potatoes and tomatoes in the field and were similar to those described by Berg (1926).

To determine if potato blight would infect tomatoes under field conditions, sprouted seed potatoes, variety International Kidney, were planted in April 1935, and when the shoots appeared above the ground on 11 May, forty-five young tomato plants were set out in rows between the potatoes. All the plants were left unsprayed. On 19 June blight appeared on the potatoes which were now fully grown and in contact with the tomatoes; on 27 June the potatoes were severely diseased, but only one spot of blight had appeared on the tomatoes; on 14 July the potato haulms were dry and shrivelled and very few diseased spots were present on the tomatoes. These tomatoes did not develop any of the small, black spots described in the above laboratory experiments.

In 1936 similar results were obtained. In each year the rapid destruction of the potatoes showed that conditions were favourable for blight. Ample time was allowed for the tomatoes to become infected, but they remained practically healthy despite the fact that they were not sprayed. Yet under field conditions tomato crops are rapidly destroyed by blight, and experience has shown that it is necessary to spray tomatoes every 7–10 days throughout the season.

The results of the laboratory and field experiments indicate that early

potato crops affected with blight are not nearly so dangerous to neighbouring tomato crops as is commonly assumed.

Inoculations with blight from late outdoor potatoes

These experiments were made in summer and autumn when both potato and tomato blight are present outdoors. The spore suspensions were obtained from diseased leaves of Kerr's Pink or Great Scot, except in 1936 when leaves from "volunteer" potato plants were used. Blight developed quickly on all inoculated potato plants; on tomatoes the following results were obtained.

In 1933 all five plants developed many small black spots. In addition, a single ordinary blight lesion, with abundant sporulation, was produced on each of two leaflets of one plant and on one petiole of another plant. This was the only time during the investigation that two distinct types of lesions were obtained on the same plant. The controls remained healthy.

In 1934 each plant developed numerous small black spots which remained small and produced many spores. Potato blight collected in autumn 1934 was grown throughout the winter on potato leaves in the laboratory by means of weekly inoculations of fresh tissue. In March and April 1935 this blight was used to inoculate tomato plants, and the results were similar to those obtained in autumn 1934.

In 1936 spore suspensions were made from diseased leaves of "volunteer" potato plants found outdoors in the autumn. Inoculation of tomato plants produced numerous small black spots which developed very few spores.

The results show that successful infection was established on all tomato plants inoculated with blight taken from outdoor potatoes in autumn. Typical tomato blight symptoms were not obtained except on two plants in 1933.

Inoculations with blight from outdoor tomatoes

In these experiments the spore suspensions were taken from diseased seedlings, leaves, stems, and fruits of outdoor tomatoes, varieties Sunrise or Devon Surprise. At this time of the season blight is usually present on both potato and tomato crops in the field.

Ten experiments were made over a period of 4 years and, in every case, typical blight symptoms developed quickly on all inoculated tomatoes and potatoes. The results agree with those recorded by Giddings & Berg (1919) and by Berg (1926).

Further trials were made to determine if blight from the infected potatoes in the above experiments would attack tomatoes. Ordinary blight symptoms developed in every case.

The results show that blight from the leaves, stems, and fruits of the tomato infected the potato, and that the blight so produced on the latter passed back readily to the tomato.

Summary and conclusions

1. In Jersey, potato and tomato crops are grown in close proximity in the open, and both suffer from severe attacks of blight (*Phytophthora infestans*). The disease appears first on the potatoes, and most growers assume that it passes from this crop to the tomatoes. To test this assumption, potato and tomato plants have been inoculated, using blight from either host plant.

2. In four out of five experiments, ordinary blight lesions developed on tomatoes inoculated with blight from glasshouse potatoes in January, February and March. The result suggests that tomato plants for the outdoor crop are liable to contract blight, if, as is often done, they are raised in glasshouses where diseased potatoes have just been, or are being, grown.

3. Blight from the early outdoor potato crop often failed to infect tomatoes. Where infection did occur typical symptoms of blight were not produced. When, under field conditions, early potatoes were interplanted with tomatoes, the former were quickly killed by blight while the latter remained almost healthy. The evidence indicates that early potato crops affected with blight are not so dangerous to neighbouring tomato crops as is commonly assumed.

4. Successful infection was established on all tomatoes inoculated with blight taken from outdoor potatoes in autumn, but ordinary blight lesions were not produced except in one experiment.

5. Blight taken from the leaves, stems, or fruits of the tomato infected the potato, and the disease so produced in the latter passed back readily to the tomato. This indicates that diseased tomato crops may be a serious menace to neighbouring potato crops.

6. The results of numerous inoculation experiments support the view that more than one strain of *Phytophthora infestans* exists.

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EXPLANATION OF PLATE X

Fig. 1. Tomato plant 14 days after inoculation with blight from potato.

Fig. 2. Tomato plant 7 days after inoculation with blight from tomato.

(*Received* 20 *August* 1937)

Fig. 1.

Fig. 2.

SMALL.—The Relation between Potato Blight and Tomato Blight (pp. 271–276)

INFECTION EXPERIMENTS WITH *CLADOSPORIUM FULVUM* COOKE AND RELATED SPECIES

By T. E. T. BOND, Ph.D.

From the Botany School, Cambridge

(With Plates XI and XII and 16 Text-figures)

CONTENTS

I. Introduction

Previous experience of the leaf-mould disease of tomatoes (*Lycopersicum esculentum* Mill.) caused by *Cladosporium fulvum* Cooke indicated that further study of the pathogenic behaviour of the causal organism was desirable (Bond, 1936); the taxonomic position of the fungus also invited a comparison with the behaviour of certain other species of the genus. *C. fulvum*, which appeared to be confined to the single host species *Lycopersicum esculentum*, nevertheless exhibited only a very slight degree of physiologic specialization (Langford, 1937). This, coupled with its ability to develop as a saprophyte, although rarely found as such under natural conditions, placed it in an interesting position between the obligate parasites, such as the rusts, showing a high degree of specialization and incapable of saprophytic development, and the relatively unspecialized, facultative parasites, such as *Botrytis* spp., which appeared

equally well fitted to a saprophytic existence as to parasitism. *Cladosporium herbarum*, according to recent opinions (Bennett, 1928; Bockmann, 1933), should be regarded as almost exclusively saprophytic, thus providing a strong contrast to the behaviour of *C. fulvum*. Various strains of *C. herbarum* were available from among the original isolations of "sooty mould" occurring on tomato foliage already infected by the leaf-mould disease. The choice of *C. cucumerinum* Ell. & Arth. as a third species for investigation was determined partly by its occurrence as a pathogen of cucumber (*Cucumis sativus* L.), apparently under conditions similar to those favourable to the development of *C. fulvum* on the tomato. As the following account will show, *C. cucumerinum* proved to be in many respects intermediate between the other two species investigated.

II. Materials and methods

Host plants

Varieties of tomato (Lycopersicum esculentum *Mill.*).

Previous work by the writer (Bond, 1936) had shown that out of a dozen common varieties "Giant Red" was the most highly susceptible, while "Stirling Castle" and "Maincrop" were relatively resistant. These three varieties were accordingly grown, seed being obtained from the same firms as formerly.

Small-fruited tomatoes.

Varieties obtained commercially included "Cascade" and the previously described "S.F. 3" type of currant tomato (Bond, 1936), identified as *L. pimpinellifolium* Mill. (= *L. racemigerum* Lange). Varieties and species obtained from other sources were as follows:

From the Royal Botanic Gardens, Kew: *L. racemigerum*.

From the University Botanic Garden, Cambridge: *L. racemigerum* (Cambridge), *L. racemigerum* Lange (from Copenhagen, No. 216–35), *L. Humboldtii* Dun. (from Copenhagen, No. 130–36).

From the John Innes Horticultural Institution: *L. pimpinellifolium* (from E. W. Lindstrom, No. 1673–3), *L. racemigerum* (Nos. 81/34 and 20/35 of Dr Sansome's collection).

The nomenclature of the currant tomatoes is discussed below (p. 287).

Other plants.

These included members of the Cucurbitaceae (Carter's "Telegraph" cucumber, *Cucumis sativus* L., also *Bryonia dioica* Jacq.), various other "inappropriate" hosts of garden origin such as asters (*Callistephus* sp.),

red and yellow snapdragon (*Antirrhinum majus* L.), and ten-week stock (*Matthiola* sp.), and the following solanaceous plants: *Solanum nigrum* L., *S. dulcamara* L., *S. melongena* L., *S. capsicastrum* Link., *Nicotiana tabacum* L. (var. "White Burley"), *N. glutinosa* L., *N. sylvestris* Speg. & Comes., *Datura stramonium* L., *D. meteloides* DC., *Atropa belladonna* L., *Hyoscyamus niger* L., *Browallia viscosa* H.B.K., *Schizanthus grahamii* Gill. In addition, the writer is indebted to Dr d'Oliveira for the following varieties of *Capsicum* grown commonly in Portugal: "Pimentos da America" (*C. annuum* L.), "Pimentos Morrones, espanhois" (*C. annuum* L., b. *grossum* Willd.), "Pimentos do Chile (Malaguetas)" (*C. frutescens* L.).

All host plants were grown from seed in potting soil supplied from the University Botanic Garden. Adequate glasshouse accommodation was available, and no difficulty was experienced in maintaining a supply of seedling plants at all times of the year except for a period of 6 or 7 weeks from the middle of November onwards.

Fungal cultures

The original cultures of *Cladosporium fulvum* (1/33) and of *C. herbarum* (2/33) were isolated in October 1933 from diseased tomato foliage obtained in the neighbourhood of Reading. Fresh single-spore isolations were made from this material at the commencement of the investigation in October 1935. Cultures received or isolated subsequently to this date were numbered as follows:

C. fulvum: 4/35 (from the Centraalbureau voor Schimmelcultures, Baarn), 10/35 (isolated from diseased tomato foliage sent from the Experimental and Research Station, Cheshunt), 11/35 (from diseased material sent from the States' Experimental Farm, Jersey).

C. cucumerinum: 2/35 (from Baarn); 1/36 (from Cheshunt).

Stock cultures of these fungi were grown on slants of Dox's medium or quaker-oat agar, incubated at 25° C. and at laboratory temperature.

Experimental methods

Inoculation experiments were carried out in the glasshouse on potted plants and in the laboratory on detached leaves in Petri dishes. The controlled environment chamber modified by Wilson (1937) was also used. For each experiment a minimum of four plants or leaves was employed, including one uninoculated control. Potted plants after incubation under bell-jars were kept free from contamination by placing them beneath light, cellophane-covered frameworks. Foliage infected

with *C. fulvum* was removed at the end of each experiment and stored in a refrigerator at 5° C. Under these conditions, the spores were found to retain their vitality for long periods. Material was inoculated by spraying with a suspension of spores in water or nutrient solution or, in some experiments, by local application of small drops of spore suspension or portions of the fungus colony, held in place by a fleck of absorbent cotton-wool. Spores of *C. fulvum* were obtained from the stored, infected foliage or from colonies on quaker-oat agar. For the other two species, colonies on Dox's agar were preferred. To prepare the spore suspensions the spore-bearing material was placed in a filter funnel and sprayed by means of an atomizer.

The details of penetration were observed in fixed material, using two different methods of examination. For sections the material was fixed in Carnoy's fluid, cleared in xylol and embedded in paraffin. Sections were cut 10–12μ. thick and were stained in carbol thionin blue and differentiated in orange G according to the method described by Stoughton (1930). Photomicrographs of these preparations were taken with the aid of a Wratten "B" (green) filter. Unsectioned material was cleared in lacto-phenol and stained in cotton blue by Pady's (1935) method. In some instances, adequate clearing was obtained only after an additional treatment with a saturated aqueous solution of chloral hydrate. Cemented mounts in lacto-phenol were found to be reasonably permanent. The preparations were photographed with the aid of a Wratten "F" (clear red) filter. The use of the lacto-phenol method permitted the examination of a much larger quantity of material than would otherwise have been possible. Moreover, the actual extent and distribution of the mycelium within the leaf could be recorded and a comparison made of the rate of penetration in different varieties and under different conditions.

III. *Cladosporium fulvum*

By most workers, the leaf-mould disease of tomatoes has been investigated largely from the practical standpoint of the discovery of control measures and the development of resistant varieties. The extensive literature dealing with the results achieved along these lines is summarized in the recent publications by Chamberlain (1932) in New Zealand, by Sengsbusch & Loschakowa-Hasenbusch (1932) in Germany, by Guba (1936) in the United States, and by Langford (1937) in Canada. The more theoretical issues of importance to the present investigation are discussed together with the relevant experimental work.

(1) *Experiments with varieties of tomato* (Lycopersicum esculentum *Mill.*)

(*a*) *Symptoms.*

Susceptible varieties. On "Giant Red", under suitable conditions, the infection is characterized by the abundant and rapid development of the fungus and the early commencement of sporulation. Infected foliage ultimately shrivels from the apex and margins inwards, becoming covered by a dense mass of spores (Pl. XI, fig. 1).

Resistant varieties. Resistance is expressed by the slower development of infection and by a reduction in the intensity of sporulation. In "Stirling Castle", the infected areas appear smaller than in "Giant Red" and remain distinct, being often localized towards the apex of the leaf. In "Maincrop", sporulation is very scanty and is confined at first to the centre of sharply circumscribed yellow blotches, where it marks, presumably, the original points of infection (Pl. XI, fig. 2). Later, as the yellowing becomes more general, the spores are produced over a wider area. In the final stage shrivelling occurs at the centre of the original blotches and also from the apex and margin inwards, as in "Giant Red". This condition, however, occurs comparatively rarely and involves only one or two leaves of the plant at a time (Pl. XI, fig. 3).

In both types the progress of infection was recorded by a numerical method adapted from that employed originally by Small (1930). For every leaflet examined, points were awarded for the visual estimate respectively of the percentage of the total area of the leaflet infected and the average degree of sporulation, as follows:

For the percentage area infected:

Up to and including 20 %	1
More than 20 %, up to and including 40 %	2
More than 40 %, up to and including 60 %	3
More than 60 %, up to and including 80 %	4
Above 80 %	5

For the degree of sporulation:

"Incipient" (no evident aerial mycelium)	1
Mycelium only (no evident sporulation)	2
Slight sporulation (pale brown)	3
Medium sporulation (dark brown)	4
Intense sporulation (dark to blackish purple) ...	5

The two marks were multiplied together to give a maximum value of 25 or (multiplied by 4) 100 %. From three to seven leaflets were recorded

on each leaf, and the total score was expressed as an average percentage value for the leaf as a whole. The same number of leaves was recorded in each plant. The results of a typical experiment involving all three varieties are presented in Table I. As the figures show, the varieties were clearly distinguishable. Infection was most severe in the middle region of the plant. At the end of the experiment the plants had grown away from the infection, leaves which to a large extent escaped infection previously becoming fully susceptible on reinoculation. The resistance of the older basal leaves was noticeable. On such leaves the early symptoms of the disease were admittedly less recognizable, but sporulation was invariably reduced. The data obtained by this method of recording infection were amenable to statistical analysis. The method was found suitable for investigating the effect of manurial treatment on the incidence of the disease. It was also used in an attempt to distinguish between the four strains of the fungus available, but with negative results. Langford (1937) has recently described from Canada a new physiologic race characterized by its extreme virulence towards varieties normally resistant to infection. Apparently, it is not known to occur in this country, in which the behaviour of resistant varieties such as, for instance, "Stirling Castle", appears fairly consistent.

Table I

Percentage intensities of infection: averages for three plants

	Leaf No.														
	(Lowest) I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	(Uppermost) XV
							"Giant Red"								
Weeks															
2	—	—	—	—	—	—	2	4	4	3	5	2	1	—	—
3	7	7	16	43	42	60	54	63	58	41	33	23	13	2	—
4	7	34	48	89	92	80	96	99	97	77	56	35	26	7	—
5	7	34	48	93	100	100	100	100	100	83	61	44	30	8	2
							"Stirling Castle"								
2	—	—	—	—	—	—	—	2	2	1	—	—	—	—	—
3	—	1	10	29	32	32	35	31	28	25	13	3	—	—	—
4	—	11	73	81	78	79	85	79	72	55	32	10	2	—	—
5	—	11	73	81	87	89	89	89	88	80	63	21	9	1	—
							"Maincrop"								
2	—	—	—	—	—	—	1	—	1	1	—	—	—	—	—
3	—	—	3	4	2	1	4	4	4	9	4	2	—	—	—
4	—	—	4	5	9	6	10	11	9	13	8	4	—	—	—
5	—	—	4	12	38	26	29	22	20	23	19	9	1	—	—

(*b*) *Histological observations.*

Penetration. Previous workers have been agreed that penetration is exclusively stomatal, and this observation is confirmed. The spores appear

equally capable of germinating in water or in moist air on the surface of the leaf. The germ tubes are slender, about $2-3\mu$. in diameter, and penetrate the stomatal aperture directly or by means of a lateral branch. No appressoria or other modifications are formed. Immediately after penetration the diameter of the hyphae appears considerably increased. The orientation of the germ tubes on the surface of the leaf is apparently haphazard, although penetration was actually observed rather more frequently in the neighbourhood of the smaller vascular bundles. Frequently the germ tubes were observed to pass across the stomatal aperture without penetration. On the other hand, penetration was only rarely prevented by the presumed closure of the stomata in darkness. Possibly the stomata retain a certain rhythm causing them to open in darkness, even if slightly and for a limited period, or perhaps closure is normally incomplete. Owing to the fineness of the hyphae, a very small residual aperture would be sufficient for penetration to occur.

Experiments were undertaken in an attempt to provide an explanation of the mechanism of stomatal penetration, and the effects of varietal differences and of differences in age or position of the leaf were investigated at the same time. The experiments were carried out in a controlled environment chamber (Wilson, 1937), the air in which was maintained at a temperature of 22·5° C. and at 85 % relative humidity. Each variety was represented by leaves taken from two different regions of the plant. The leaves were numbered similarly to those in the experiment recorded in Table I, and were at a similar stage of development. The three apical leaflets were inoculated in each case. These latter were placed in Petri dishes containing moistened filter paper and were inoculated (on the lower surface) with a standard spore suspension containing the spores from a 2-month colony on quaker-oat agar to 5 c.c. of sterile water. Each experiment was in two series; in one the lids of the dishes were left in position, giving constant saturation, while in the other the lids were removed during the day and replaced at night, giving a fluctuation between saturation and 85 %, the humidity of the air in the chamber. The filter paper in the dishes was moistened when necessary. After 5 days, the numbers of penetrating hyphae were recorded over equal areas removed with the aid of a cork borer 1·4 cm. in diameter and examined by the lacto-phenol method. The experiments are summarized in Table II, the figures showing that, in a total of over six hundred instances, penetration was roughly 7·5 times more frequent in the "fluctuating" series than at constant saturation. That this was not due merely to the evaporation of the water droplets and the consequent

removal of the mechanical barrier created by their surface tension, in the former series, was shown by the fact that a similar result was obtained in an experiment in which both series of dishes, before transferring to the chamber, were left uncovered on the bench until all traces of moisture on the surface of the leaf had evaporated. It is noticeable that the results of Exp. XLIII, in which the chamber was maintained in darkness, are in no way unusual. The evidence as a whole points to the conclusion that penetration is controlled largely by a stimulus depending on evaporation from the leaf. Least difficulty is involved in the assumption that the stimulus is actually hydrotropic, and further circumstantial evidence in support of this is provided in the following sections. In Exps. XLV and XLVI, the results were analysed for differences between varieties and between leaf ages respectively (Table III). In each case, the frequency of penetration was considerably lower in "Maincrop" than in "Giant Red". As regards the different leaf ages, the results of the second experiment only were significant (i.e. to the 5% level of probability), penetration being less frequent in the upper position. This might account for the

Table II

Summary of measurements of the frequency of penetration in relation to humidity

Exp.	Variety	Position of leaf	No. of leaflets examined	Nos. of hyphae penetrating in equal areas under different conditions: "Constant"	"Fluctuating"
XLI	GR, M	VII only	12	39	137
XLII	GR only	Do.	6	—	18
XLIII	GR, M	Do.	12	21	85
XLV	GR, M	III, VII	24	4	175
XLVI	GR, M	VI, XI	24	8	114
XLVII	GR only	VII only	6	1	21
Totals:			84	73	550
Averages for each series:				1·7	13·1

GR and M respectively represent the varieties "Giant Red" and "Maincrop".

Table III

Summary of measurements of the frequency of penetration in different varieties and in different regions of the plant

(Numbers of hyphae penetrating in equal areas)

Exp.	"Giant Red" Lower	Middle	Upper	"Maincrop" Lower	Middle	Upper
XLV	92	46	—	17	24	—
XLVI	—	54	18	—	35	15
	210			91		

extreme localization of infection frequently seen in the upper leaves (see Pl. XI, fig. 2). In the uppermost leaves, corresponding to Nos. XIV–XV in these experiments, penetration was rarely observed, but this may be accounted for by the fact that at this stage the stomata are extremely small and undeveloped, the majority being apparently non-functional.

Initial development of mycelium. The penetrating hyphae produce an extensive intercellular mycelium, from which no haustoria are developed. Growth is characterized by the production of long, straight "runners", passing between the cells of the spongy mesophyll and sending out further ascending branches at intervals (Text-figs. 1, 2). The hyphae are approximately 4–5μ. in diameter and are typically septate, densely granular and non-vacuolate. They show an increasing tendency to become localized in the vascular region (Text-fig. 4) but were not actually observed in contact with the tracheids, since they are apparently unable to penetrate between the cells of the surrounding parenchyma (Pl. XI, fig. 4). Measurements illustrating the development of an internal mycelium in susceptible and resistant varieties are summarized in Table IV. Comparison of the rate of spread of mycelium under different conditions is complicated by the steady increase in the total number of penetrating hyphae observed on successive days. Nevertheless, the results expressed in Table V indicate a strong tendency for the rate of spread to be greater in "Giant Red" than in "Maincrop". The measurements in Table V were made at random over equal areas of the leaf in each experiment. A difference of more than three times the standard deviation is held to indicate significance. According to the analysis of the results of Exp. XLV, the rate of penetration is also significantly lower in the lower leaves (No. III) than in the middle of the plant (No. VII). Detailed measurements for the upper leaves in Exp. XLVI were not available owing to the reduced frequency of penetration in this position (p. 284).

Table IV

Initial development of mycelium in susceptible and resistant varieties. (Equal areas examined)

	"Giant Red"		"Maincrop"		
Days	No.	Av. diam. μ.	No.	Av. diam. μ.	Total no. of penetrating hyphae
1	1	15	—	—	1
2	5	47	4	26	9
3	17	44	11	51	28
6	45	751	46	653	91

The diameter is that of the minimum circle within which all the hyphae from any one stoma may be included.

Table V

Comparative rate of spread of mycelium

Exp.	Days from inoculation	Total no. of measurements	Position of leaf	Av. diam. of mycelium μ. GR	M	Standard deviation
XI	6	50	VII	763	532	68
XLV	5	32	III	458	142	23
		32	VII	653	444	91*
XLVI	5	48	VI	305	165	40

* The difference is less than three times the standard deviation.

Sporulation. Under favourable conditions, sporulation commences after 10–11 days. The conidiophores originate from stromatic bodies formed in the substomatal cavities and they emerge through the greatly enlarged stomatal aperture (Pl. XI, fig. 5). If sporulation is prevented or delayed by unfavourable conditions, the stromata remain sterile while increasing in size. Eventually, the epidermis may be ruptured and occasionally a few short aerial hyphae are produced (Pl. XII, fig. 1). During this period, the hyphae themselves increase in diameter and become vacuolated. The mycelium as a whole becomes very much more abundant and stromatic aggregations develop also around the vascular bundles, many of which become completely enveloped in a sheath of hyphae.

Final stages. Yellowing of the foliage in the later stages of infection is commonly associated with the occurrence of necrosis affecting groups of cells, particularly in the palisade region. The condition is especially evident at the centre of the localized lesions on "Maincrop", and it appears to result from a withdrawal of water by the abundant development of the mycelium. The associated hyphae are swollen and highly vacuolated. Later, when the supply of food materials in the leaf is seriously diminished, the hyphae tend to become lobed and distorted (Pl. XII, fig. 2). In the final stage, after the death of the cells, the walls are broken down and the mycelium consequently appears intracellular. Intra- as well as intercellular mycelium was described by Makemson (1918) and by Chamberlain (1932), but according to the present observations the fungus should be regarded as strictly intercellular. *Cladosporium fulvum* appears to flourish in the leaf only so long as the tissues are alive; after the death of the cells, only a limited and in some ways abnormal development can occur.

(2) *Experiments with other* Lycopersicum *species*

No symptoms were recorded on any of the small-fruited tomatoes inoculated with the exception of *Lycopersicum Humboldtii* Dun., from Copenhagen, and the two strains received as *L. racemigerum*, from the John Innes Horticultural Institution. These had previously been reported immune (Bond, 1936). In several respects, they appeared closer to the cherry tomato, *L. cerasiforme* Dun., than to the true currant tomato. The latter was reported immune to *Cladosporium fulvum* by Sengsbusch & Loschakowa-Hasenbusch (1932), Osmun (1934), Alexander (1934), Guba (1936), Langford (1937), and previously by the writer (1936). The species is variously described as *Solanum racemigerum* (Sansome, 1933) or as *Lycopersicum racemigerum* Lange (= *L. racemiforme* Lange), also as *L. pimpinellifolium* Dun. (Bailey, 1922; Lindstrom, 1932) and *L. pimpinellifolium* Mill. (Guba, 1936). The latter is preferred on grounds of priority. The genetic basis of immunity in the currant tomato has recently been elucidated by Langford (1937), whose work confirms and extends that of previous investigators. In addition to the factor controlling immunity, a second factor occurs which confers a degree of partial resistance. This latter, however, is not identical with the factor for resistance in the varieties of *L. esculentum*. Schmidt's (1933) theory of the nature of immunity in the currant tomato is unfortunately untenable, having been founded on a misconception as to the identity of the causal organism (Raabe & Sengsbusch, 1935).

Histological observations. Langford (1937) described the occurrence of stomatal penetration in the currant tomato, and this observation is confirmed. Penetration occurs in the same manner and apparently with the same readiness as in the commercial varieties. Moreover, the effect of humidity on the frequency of penetration was also similar, totals respectively of 68 and 189 penetrating hyphae being recorded for the "constant" and "fluctuating" series in Exps. XLI-XLIII, in which the "S.F. 3" currant tomato was inoculated together with the varieties "Giant Red" and "Maincrop" (see p. 283). The effect was equally noticeable in leaflets inoculated and maintained in darkness. The mycelium after penetration is intercellular and without haustoria. Its extent appears to depend on the relative maturity of the leaf, which in turn is influenced by cultural conditions and the season of the year. In immature leaves and in young leaves developed during the winter months (thin, "shade" leaves with abundant intercellular spaces), it rapidly becomes stoloniferous to a degree comparable with that observed

in the early stages on "Giant Red" tomato. The host cells appear unaffected by the presence of the hyphae. Beyond this stage, however, no further development occurs. A gradual increase in diameter may take place, but the long runners remain comparatively scattered and far apart, not giving rise to any stromatic aggregations (Text-figs. 3, 4). The maximum length of time during which the mycelium remains alive under these conditions was not determined. The development of an extensive, stoloniferous mycelium takes place only under the conditions described. As the leaves become older, and in leaves developed under progressively better conditions, the mycelium is more and more restricted. At the same time there is a tendency for the host cells in immediate contact with the hyphae to become necrotic. In general, only isolated cells were affected and no external symptoms were produced. Langford (1937), however, observed a flecking of the foliage after inoculation in five only out of more than a hundred experiments. In the final stage, the mycelium is greatly restricted, less than 100μ. in diameter, and in this condition frequently becomes excessively branched (Text-fig. 5). Measurements illustrating this course of events are summarized in Table VI. The figures are intended to be representative only; the records always showed great variability and no statistical analysis was attempted.

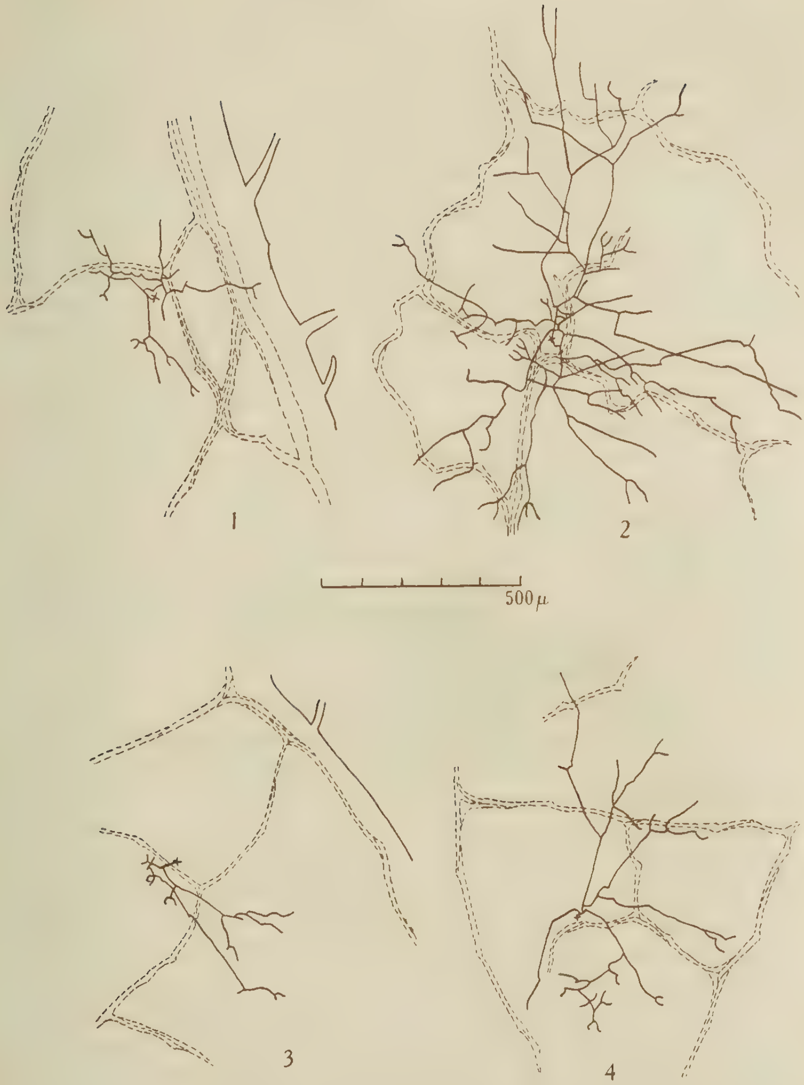
Table VI

Rate of spread of mycelium in foliage of currant tomato at successive stages of development

Stage	Description	Days from inoculation	No. of measurements	Mycelium Av. diam. μ.	Mycelium Maximum μ.
A	First and second leaves of seedlings, in February	4	14	400	640
			18	350	760
		6	3	215	340
			6	439	735
		10	2	435	640
		17	7	320	860
B	Young leaves, March–April	1	1	10	10
		2	2	33	41
		3	3	75	150
		6	8	180	300
			7	150	225
C	Mature leaves, May–June	6	6	58	90

(3) *Experiments with immune and "inappropriate" hosts*

No external symptoms of infection were recorded on any of the plants inoculated and all, by this criterion, were entirely immune. Similar failures to extend the host range of the fungus were reported by Sengsbusch & Loschakowa-Hasenbusch (1932), Guba (1936), and other



Text-figs. 1–4. Course of penetrating hyphae. (These and subsequent text-figures are from lacto-phenol preparations, viewed from the lower surface of the leaf, and drawn with the aid of a camera lucida.)

Text-fig. 1. "Giant Red" tomato, after 4 days.

Text-fig. 2. "Giant Red" tomato, after 7 days.

Text-fig. 3. "S.F. 3" currant tomato, after 6 days (stage "A").

Text-fig. 4. "S.F. 3" currant tomato, after 17 days (do.).

workers. *Cladosporium fulvum* was recorded as a pathogen of *Solanum melongena* by Ciferri (1927), in the Dominican republic, but the same author later (1930) stated that the disease was seldom seen and was not clearly identified. Pritchard & Porte (1921) also noted a closely similar fungus infecting *S. carolinense* in the eastern United States, but were again unable to confirm its identity by inoculation experiments.

Histological observations. Examination of inoculated, fixed material of the apparently immune plants revealed the ready occurrence of stomatal penetration in all species with the exception of 10-week stock (*Matthiola* sp.), in which the epidermis on both surfaces of the leaf is protected by a dense covering of stout, branched hairs. On the other plants the penetrating hyphae pass directly through the stomatal aperture in the manner previously described for *Lycopersicum esculentum*. Penetration is not normally prevented by the presumed closure of the stomata in darkness. Throughout the experiments as a whole, penetration appeared less frequent than in susceptible hosts. Thus, in a whole series of inoculations carried out under uniform conditions and involving approximately equal areas of foliage, the average frequency of penetration per sq. cm. was 14·5 for three tomato varieties and 7·1 for ten other solanaceous plants. In some instances, however, this relationship was appreciably modified, or even reversed. In each of two experiments involving both *Nicotiana tabacum* and *Antirrhinum majus*, the frequency of penetration was higher at fluctuating humidity than at constant saturation, the difference being significant at the 5 % level of probability (see p. 283). This suggests a mechanism of penetration similar to that which is effective in susceptible hosts. The internal mycelium is uniformly intercellular and without haustoria but it varies greatly in extent in the different species and under different conditions. Wherever possible the diameter of penetration was recorded 1, 2, 3, and 6 days after inoculation and again after an interval of about 4 weeks. In some plants the series of observations was less complete. The results as a whole seemed to fall into three groups, which are discussed separately.

The first group of results (Table VII) comprises eight different solanaceous plants in which the maximum diameter of penetration after 6 days was 200μ. or greater. As in the currant tomato, the extent of the mycelium varies with the age or condition of the foliage, and this is illustrated by the grouping of the results in three columns "A", "B", and "C", corresponding to the three stages recognized in Table VI. In some plants, for instance *Solanum melongena*, these represented fairly well-marked morphological stages in the development of the leaf, but in

no case could any absolute distinction be drawn. The maximum diameter of penetration is associated with young foliage, developed early in the year and under reduced light intensity, thin, and with abundant intercellular spaces. In this condition the mycelium is frequently stoloniferous, often with subepidermal hyphae following the grooves marking the inner edge of the radial walls of the epidermal cells (Text-fig. 6). The host cells appear but little affected by the parasite, and such response as was noticed (chiefly discoloration and presumed eventual necrosis) was comparatively slight and infrequent. Similarly, the fungus mycelium is normal in appearance and is checked only in the vigour of its development, suggesting a deficiency of some necessary food material. As the cells of the leaf become better developed, the diameter of penetration is reduced and necrosis appears frequently, probably on account of the greater closeness of contact between the host cells and the invading hyphae. Finally, the mycelium is restricted to one or two short branches between the cells immediately adjacent to the stomatal cavity (Text-figs. 7, 8) or even to a few swollen, peg-like outgrowths actually in contact with the inner face of the guard cells. If this condition is associated with necrosis, it is sometimes impossible to determine whether the hyphae have actually passed the stomatal aperture or not.

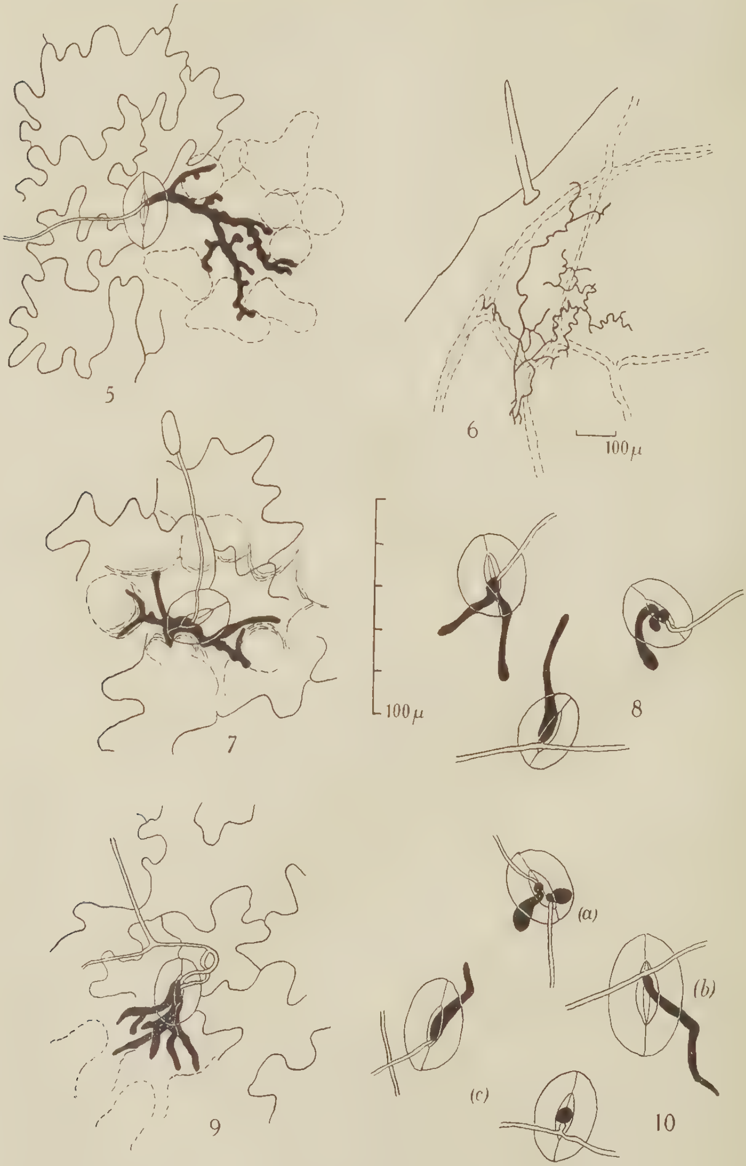
Table VII

Rate of spread of mycelium in eight other solanaceous hosts
(Number and extent of penetrating hyphae)

	"A"			"B"			"C"		
Days from inoculation	No.	Av. diam. μ.	Max.	No.	Av. diam. μ.	Max.	No.	Av. diam. μ.	Max.
1	2	25	25	—	—	—	—	—	—
2	18	38	110	—	—	—	—	—	—
3	69	85	450	15	39	125	—	—	—
6	103	215	1020	66	50	90	12	32	70
25–29	1	650	—	91	69	300	107	36	90

The following species are represented: *Solanum melongena, S. dulcamara, Schizanthus grahamii, Atropa belladonna, Hyoscyamus niger, Nicotiana tabacum, N. sylvestris, N. glutinosa.*

The second group (Table VIII) includes species in which the maximum diameter of penetration was more than 100μ., but did not exceed 200μ. after the first 6 days. Leaves in the juvenile or "A" condition were apparently comparable in every respect with those of species previously considered, in which the development of mycelium was usually far more extensive. The diameters attained, however, were more comparable with



Text-figs. 5–10.

those recorded for the "B" condition in the previous table. Consequently, the figures are considered in two groups only, "A" and "B" (Text-fig. 9).

Table VIII

Rate of spread of mycelium in four other solanaceous hosts
(Number and extent of penetrating hyphae)

	"A"			"B"		
Days from inoculation	No.	Av. diam. μ.	Max.	No.	Av. diam μ.	Max.
2	3	27	35	—	—	—
3	27	35	75	—	—	—
6	89	53	145	12	17	30
25–29	5	250	300	73	19	85

The following species are represented: *Solanum capsicastrum, S. nigrum, Datura stramonium, Browallia viscosa.*

In the remaining species, including the Capsicums and the "inappropriate" hosts belonging to other families, penetration was even more reduced. The maximum diameter after 6 days was that of 110μ. in *Callistephus* sp. In *Antirrhinum majus* and *Bryonia dioica*, however, penetration was not observed to exceed 50μ. even in the youngest leaves, the mycelium being confined typically to single, peg-like or slightly swollen branches (Text-fig. 10). In contrast to the species included in the two previous groups this restriction in the development of mycelium is rarely associated with necrosis, thus indicating the possible existence of a factor initially unfavourable to the fungus, not depending on an active reaction between the host cells and the invading hyphae.

IV. *Cladosporium cucumerinum*

Cladosporium cucumerinum Ell. & Arth. is known in this country as the cause of cucumber "gummosis", which is a disease largely confined to the fruits. In the United States it is also known to cause a

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Text-fig. 5. Limited mycelium in "S.F. 3" currant tomato, after 6 days (stage "C": mature).

Text-fig. 6. Extensive mycelium in *Solanum melongena*, after 18 days, showing subepidermal hyphae in centre (stage "A").

Text-fig. 7. Limited mycelium in *Hyoscyamus niger*, after 6 days, showing ring of discoloured cells (stage "C").

Text-fig. 8. Short hyphae in *Solanum melongena*, after 29 days, not associated with discoloured cells (stage "C").

Text-fig. 9. Limited mycelium in *Solanum nigrum*, after 6 days (stage "A").

Text-fig. 10. Penetration in "inappropriate" hosts, after 6 days, not associated with discoloration.

(*a*) *Antirrhinum majus*, (*b*) *Bryonia dioica*, (*c*) *Callistephus* sp.

destructive disease of the foliage and seedling plants, referred to originally as "leaf blight" (Humphrey, 1892) or more recently as "scab" (Doolittle, 1916). A similar condition has been reported from Holland (Pfältzen, 1927; Muyzenberg, 1932) and other countries (Eriksson, 1915). According to v. Höhnel (1923), the fungus *Scolecotrichum melophthorum* Prill. & Del., causing "La Nuile" of melons (Prillieux & Delacroix, 1891) and cucumbers (Delacroix, 1892) in France is identical with *Cladosporium cucumerinum*. This synonymy was upheld by Garbowski (1924) and was later confirmed by the inoculation experiments of Pfältzen (1927). In England the fungus appears to have been described first by Cooke (1903, 1903*a*), who named it *C. scabies* Cke. Both strains of *C. cucumerinum* investigated produced extensive fertile colonies on both Dox's medium and quaker-oat agar, growth being only a little slower than in *C. herbarum* (Pl. XII, fig. 3). Consequently, they were readily distinguishable in appearance from *C. fulvum*. Colonies of strain 1/36 (from Cheshunt) were generally paler in colour than those of strain 2/35 (from Baarn), and the spores were larger in all dimensions. Spore dimensions of *C. cucumerinum*, *C. fulvum*, and *C. herbarum* are summarized in Table IX.

Table IX

Spore measurements for three species of Cladosporium

		One-celled		Two-celled
C. fulvum		(89%)		(10%)
(Average of 4 strains)	(200)	$16{\cdot}2 \pm 0{\cdot}26 \times 6{\cdot}8 \pm 0{\cdot}07$	(200)	$26{\cdot}9 \pm 0{\cdot}41 \times 6{\cdot}6 \pm 0{\cdot}08$
C. cucumerinum		(79%)		(21%)
1/36	(50)	$15{\cdot}2 \pm 0{\cdot}73 \times 5{\cdot}0 \pm 0{\cdot}13$	(25)	$22{\cdot}5 \pm 1{\cdot}00 \times 5{\cdot}7 \pm 0{\cdot}17$
2/35	(50)	$11{\cdot}8 \pm 0{\cdot}44 \times 4{\cdot}1 \pm 0{\cdot}11$	(25)	$16{\cdot}4 \pm 1{\cdot}05 \times 4{\cdot}6 \pm 0{\cdot}26$
C. herbarum		(96%)		(4%)
	(50)	$4{\cdot}7 \pm 0{\cdot}18 \times 3{\cdot}5 \pm 0{\cdot}09$	(10)	$15{\cdot}0 \pm 0{\cdot}70 \times 4{\cdot}9 \pm 0{\cdot}26$

Dimensions are in μ.

Spores of *C. fulvum* were obtained from "Giant Red" tomato foliage, 17 days after inoculation.

Spores of *C. cucumerinum* and of *C. herbarum* were obtained from colonies on Dox's medium, after 3 weeks at 25° C.

(*a*) *Proof of pathogenicity*

Inoculations were carried out on seedling plants and detached leaves of Carter's "Telegraph" cucumber and of *Bryonia dioica*, also on cucumber fruits (variety unknown) and on plants and leaves of the following "inappropriate" hosts, namely, "Giant Red" and "Maincrop" tomato, the "S.F. 3" currant tomato, *Solanum nigrum*, *Antirrhinum majus*, *Callistephus* sp. Both strains were pathogenic to cucumber fruits. Needle inoculation with spores removed from a colony on Dox's medium

produced sunken lesions attaining a diameter of about 1·5 cm. after the first 8 days (Pl. XI, fig. 4). Sporulation was evident after the second day, the lesions being similar in colour to the colonies on Dox's medium. Frequently they were surrounded by a yellowed or paler area. No trace of gum exudation was observed on these lesions, and van der Muyzenberg (1932) had previously noted a similar absence of gummosis in certain instances. Similar lesions were observed repeatedly after reisolation and subsequent inoculation, the reisolates also retaining the cultural characteristics of the parent strains. Inoculations with *Cladosporium herbarum* and with the *C. fulvum*, carried out under similar conditions, gave negative results, a very scanty growth of the fungus being observed only at the actual point of inoculation. Spore germination by *C. cucumerinum* in distilled water was unsatisfactory and for spray inoculations the spores were usually applied in half-strength Dox's solution, using the same solution for the control experiments. On cucumber seedlings infection was severe, but the symptoms were confined to the basal and apical regions of the plant and were rarely so extensive as those described by Humphrey (1892) and later by van der Muyzenberg (1932). The apex was usually destroyed, and on the lower leaves large, water-soaked lesions developed, with abundant sporulation. Small necrotic lesions occurred on the stems, petioles, and veins of the leaves; these were frequently associated with a drop of gummy exudation. From the results available no distinction could be drawn affecting the pathogenicity of the two strains investigated. Strain 2/35 was also found to cause the destruction of the young stems, leaves and tendrils of *Bryonia dioica*, but no symptoms were observed on any other plant inoculated.

(*b*) *Histological observations*

Cucumber fruits. In the early stages of infection, the hyphae are many-septate and irregularly swollen, about 8–10μ. in diameter. They penetrate the cell walls readily and apparently by mechanical pressure, no evidence of enzyme action being observed. The cells in advance of the hyphae and those in lateral contact with them appeared unaffected. No special aggregations of hyphae are formed in the neighbourhood of the vascular bundles. The mycelium penetrates into actual contact with the xylem elements, many of which are invaded by hyphae passing between the thickened spirals. Ultimately, the outer tissues of the rind are destroyed and the mycelium becomes consolidated to form a dense, superficial crust, 100μ. or more in thickness. Afterwards, there is probably a more limited and chiefly intercellular development in the

lower layers, in which the cells are larger and have thicker walls more able to resist penetration (Pl. XII, fig. 5).

Cucumber foliage. Treatment with chloral hydrate was necessary to ensure adequate clearing (p. 280). Penetration is stomatal, the process being similar to that previously described for *Cladosporium fulvum.* The resultant mycelium is initially intercellular, without haustoria. The hyphae early become swollen, reaching a breadth of 8–10μ., but they are not distorted as they are in the fruits. The growth of the mycelium is sometimes very rapid, a maximum extent of 350μ. having been recorded 3 days after inoculation. Branching is characteristically dendritic (Text-fig. 11). The long, straight runners typical of the mycelium of *C. fulvum* on tomato are not formed and there is no tendency to the formation of stromatic aggregations enclosing the vascular bundles. The exact period at which death of the host cells occurs was not determined. In some leaves the growth of the mycelium is arrested before any effect on the host is noticeable. Occasional sections, cut 10 days after inoculation, showed the mycelium still apparently intercellular, with single conidiophores emerging through the stomata. The more normal condition is for sporulation to occur only in those parts of the leaf previously killed by the parasite, when the mycelium is apparently intracellular, with the conidiophores passing directly through the remains of the epidermal walls.

"*Inappropriate*" *hosts.* Normal stomatal penetration was observed repeatedly on all other plants inoculated, with the following maximum diameters of penetration 6 days after inoculation: "Giant Red" tomato 50μ., "Maincrop" tomato 45μ., "S.F. 3" currant tomato 85μ., *Solanum nigrum* 10μ., *Antirrhinum majus* 80μ. (Text-fig. 12). Penetration is not usually associated with necrosis or discoloration of the cells. The hyphae are frequently unbranched, ascending rapidly towards the palisade; subsequent measurements failed to reveal any significant increase in

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Text-fig. 11. *C. cucumerinum* in foliage of *Cucumis sativus*, after 8 days.

Text-fig. 12. *C. cucumerinum* in *Antirrhinum majus*, after 6 days.

Text-fig. 13. *C. cucumerinum* in *Antirrhinum majus*, "peg"-like outgrowths, after 25 days, in older foliage. (*a*) *Lycopersicum esculentum*, (*b*) *Solanum nigrum*, (*c*) *Antirrhinum majus*.

Text-fig. 14. *C. herbarum*, extensive mycelium in currant tomato, after 6 days, young condition.

Text-fig. 15. *C. herbarum* in *Solanum nigrum*, young condition, showing internal sporulation.

Text-fig. 16. *C. herbarum* in *Solanum nigrum*, limited mycelium in mature foliage, associated with necrosis.

Text-figs. 11–16. *C. cucumerinum* and *C. herbarum.*

extent after the 6-day period. In older leaves the mycelium rarely develops beyond one or more swollen, peg-like outgrowths, similar to those produced under comparable conditions by *Cladosporium fulvum*. These appeared unchanged after an interval of 25 days from inoculation (Text-fig. 13).

Frequency of penetration. The effect of humidity on the frequency of penetration was investigated by the method described for *C. fulvum* (p. 283). In each of two experiments, involving "Telegraph" cucumber, "Giant Red" tomato, *Solanum nigrum*, and *Antirrhinum majus*, the frequency of penetration was higher at "fluctuating" humidity than at constant saturation, but the difference failed to reach significance at the 5 % level of probability. No conclusions as to the mechanism controlling penetration can be drawn from these experiments. Considering the relatively high concentration of spores in the suspensions used for inoculation, and their abundant germination, the frequency of penetration at either condition of humidity was always surprisingly low. The possibility remains that such penetration as there is occurs entirely by chance, although the influence of such factors as the age of the spores and the conditions of germination must not be overlooked. Limited observations with spores germinated in distilled water indicated that the use of a nutrient solution to ensure adequate germination had no effect on the subsequent course of events described above.

V. *Cladosporium herbarum*

C. herbarum (Lk.) Fr. is well known as a ubiquitous saprophyte (Brooks & Hansford, 1923; Fraser, 1934), although from time to time various parasitic activities have been attributed to it. These reports were summarized by Bennett (1928), who showed that the fungus should be regarded as a saprophyte capable of becoming "semi-parasitic" only on moribund tissues. A similar conclusion was reached later by Bockmann (1933). No evidence of pathogenicity was observed by the present writer. Inoculations of seedling plants and detached leaves of "Giant Red" and "Maincrop" tomato, "S.F. 3" currant tomato, *Solanum nigrum*, and *Antirrhinum majus* gave negative results, even when the spores were applied in a nutrient solution, to ensure adequate germination.

Histological observations. Stomatal penetration was observed in all plants inoculated, the germ tubes passing through the stomatal aperture in the manner described for the other two *Cladosporium* species. A limited mycelium is produced, which is intercellular and without haus-

toria. The mycelium differs considerably in appearance and extent according to the species and the age of the foliage inoculated. In the tomato varieties, it is confined to one or several peg-like outgrowths not more than 75μ. in extent, usually without any evident reaction of the host cells. A similar appearance was observed in the currant tomato, in which plant, however, the mycelium on one occasion reached an extent of 125μ. without necrosis (Text-fig. 14). In *Solanum nigrum*, the mycelium in young, poorly developed foliage consists typically of a single, unbranched hypha and occasionally, internal condidiophores are developed (Text-fig. 15). These were not observed on any other species. In older foliage of *S. nigrum*, penetration is usually associated with necrosis, the limited mycelium being swollen and distorted (Text-fig. 16). Possibly, this condition results in part from the confined dimensions of the stomatal cavity, which may become partly "occluded" by the growth of the surrounding mesophyll cells. Only a limited development of mycelium was recorded in *Antirrhinum majus*. In a single experiment involving all three host species, the frequency of penetration was slightly higher at constant saturation than at "fluctuating" humidity (see p. 283), but the difference was not significant to the 5% level of probability. In general, the frequency of penetration was of the same order as that observed in *Cladosporium cucumerinum*, i.e. considerably below that of *C. fulvum* under similar conditions.

VI. Discussion

Brown (1936, p. 265) has recently expressed the opinion that "the relation of the mycelium of the parasite to the host cell gives one of the best criteria for distinguishing the two types...", i.e. of obligate or facultative parasitism. The same author also refers to *Cladosporium fulvum* and some other fungi as representing the class of "facultative saprophytes" rather than "facultative parasites". These statements form a convenient basis for a discussion of the observations contained in the present communication.

The mycelium of *C. fulvum* is strictly intercellular. There are no haustoria, which are characteristic of the obligate type of parasite, and there is no evidence for any enzyme action enabling it to penetrate between host cells which are normally in contact. Apparently, the host cells may later be separated to a certain extent by the continued growth and aggregation of the hyphae in the original intercellular spaces. The growth of the fungus appears to depend on the maintenance of a sufficiently close contact between host and parasite. This is seen particularly

in the sheath of hyphae surrounding the vascular bundles and, in the earlier stages, in the short, clasping hyphae attached to the free ends of the palisade cells. As to how far this contact may be compared with the union effected by a haustorial parasite remains uncertain. The appearance suggests an active withdrawal of water and dissolved food substances from the host cells rather than the mere utilization of the products of normal exosmosis. The absorption of water would presumably depend on a simple osmotic relationship, but the passage of dissolved substances would require an additional mechanism involving possibly an active influence exerted by the fungus on the permeability of the protoplasm of the host cells. Death of the host cells is accompanied by abnormalities in the associated hyphae which become progressively swollen and distorted and make no further active development. In this respect a strong resemblance is shown with the obligate type of parasite. However, there is no obvious mechanism of resistance comparable with the "hypersensitive" reaction to certain rust fungi. On the other hand, even in the earliest stages of infection the rate of spread of mycelium is less in the resistant varieties, just as it is in the relatively resistant lower leaves of an individual plant. That the factor for varietal resistance is a relatively simple one is suggested by its inheritance as a single Mendelian character (Guba, 1936). However, the existence of so many degrees of resistance among the different varieties suggests that a quantitative reaction may be involved. The behaviour of Langford's (1937) recently described physiologic race, which is reported to produce "susceptible" symptoms on varieties resistant to the normal strains, seems to lend further support to the view that resistance results from some relatively simple character. Langford's mutant strains, producing necrotic non-sporing lesions on resistant and susceptible types alike, recall the sterile variants originally described by Caldis & Coons (1927). Similar variant strains have been isolated by the present writer. The peculiar type of lesion produced appears to result from the initially more rapid development of the sterile strains (as compared with the fertile types), leading to their own arrest through the premature exhaustion and death of the host cells. The mechanism of stomatal penetration by *C. fulvum* on tomato was discussed in connexion with the experiments described on p. 283. The writer agrees with Brown (1936, p. 245) that "no difficulty is involved in assuming that a germ tube grows accidentally over one stoma or another". The experiments indicate that "the second stage of the process" (*loc. cit.*), that is the actual initiation of penetration by the apex of the germ tube or by a newly developed lateral branch, is controlled in

part by a stimulus depending on the evaporation of water from the leaf. The fact that similar results were obtained with immune and inappropriate hosts suggests that the stimulus to penetration is hydrotropic, i.e. entirely non-specific to the susceptible hosts. The experiments recorded are believed to be the first in which evidence as to the mechanism of stomatal penetration has been obtained from the use of controlled conditions of humidity affecting the frequency of penetration as it occurs on the host plant. The only previous work of significance on this point, by Balls (1905), involved the use of artificial membranes of perforated rubber.

In view of the above conclusion the problem of the invasion of immune and inappropriate hosts is primarily that of accounting for the loss of pathogenicity and of explaining the differences in extent and distribution of the mycelium in the different species. Similar questions were raised by the experiments of Gibson (1904), Young (1926), Johnson (1932), Corner (1935), Hanes (1936), and other workers, although the organisms investigated differed from *C. fulvum* either by the formation of appressoria for stomatal or direct penetration or by the haustorial habit of their mycelium. In the currant tomato the distribution of the internal mycelium of *C. fulvum* depends largely on the age and structure of the particular leaf inoculated. The extensive mycelium produced in the young, poorly developed leaves is apparently parasitic to a certain extent. Possibly, immunity at this stage will prove closely similar to resistance in "Maincrop" tomato, the two types of reaction differing only in degree. In the adult condition this difference might become more pronounced, but the frequent occurrence of an active necrotic reaction of the host cells would appear to represent an additional mechanism of a different type. Indirect evidence of this may possibly be deduced from the existence of two independent factors controlling resistance and immunity respectively in Langford's (1937) *pimpinellifolium* × *esculentum* crosses. In at least eight other solanaceous species, from five different genera (Table VII), the course of events is essentially the same as in the currant tomato. In other species (Table VIII) a somewhat reduced diameter of penetration can be correlated with certain structural or histological peculiarities. The case of *Solanum nigrum* has already been mentioned (p. 299). *Browallia viscosa*, again, appears to have unusually small cells with poorly developed intercellular spaces. Finally, in the Capsicums and in the plants belonging to other families, the mycelium remains restricted even in the young foliage, although usually without any necrotic reaction of the host cells. The observations recorded admit

the possibility that immunity in the currant tomato and in many other Solanaceae may be genetically related, while in the last group of plants mentioned there would appear to exist genuine and entirely unrelated "non-susceptibility" to *Cladosporium fulvum* (Reddick, 1928), the nature of which remains unsolved.

C. cucumerinum and *C. herbarum* agree closely with *C. fulvum* in their manner of stomatal penetration and in the habit of the resultant mycelium. Where the mycelium of *C. cucumerinum* is intracellular, in the thin-walled rind tissues of the cucumber, the hyphae appear to penetrate the cell walls by mechanical pressure. In the thicker walled inner layers they are more frequently confined to the intercellular spaces. The behaviour of *C. cucumerinum* on cucumber foliage differs from that of *C. fulvum* on tomato chiefly in the fact that sporulation is largely delayed until the host cells are dead. This recalls its more ready growth on artificial media and indicates that it should be classified as a true facultative parasite. The spores are also smaller than those of *C. fulvum* and are less able to germinate in the absence of nutrients. On "inappropriate" hosts, the mycelium is not usually associated with necrosis of the host cells. An interesting feature is the possibility of the existence of distinct strains showing different pathogenic activities, particularly as regards their virulence to the foliage and seedling plants. The behaviour of *C. herbarum* in the present investigation confirms the generally accepted opinion of Bennett (1928) and other workers, that it is essentially saprophytic. On all plants inoculated the mycelium remains limited in extent and gives no indication of parasitic activity.

The question arises as to how far the pathogenic activity of the three species investigated will prove to be characteristic for the genus as a whole. The most important features under discussion can be summarized as follows: (1) stomatal penetration by unaltered germ tubes, (2) mycelium in the foliage typically intercellular, not producing haustoria, (3) conidiophores essentially subepidermal in origin, not forming superficial or subcuticular layers. In one species at least, namely, *C. paeoniae* Passer, this behaviour is probably closely paralleled. According to Meuli's (1937) recent account the mycelium of this species is at first largely superficial, with occasional stomatal penetration. Eventually, after the death of the host tissues (which occurs in a manner still unexplained) an abundant internal mycelium is observed, with conidiophores emerging through the stomata. The third character mentioned, namely, the mode of origin of the conidiophores, may possibly be useful in determining the true systematic position of the two species *C. carpo-*

philum Thüm and *C. effusum* (Wint.) Demaree, causing "scab" of stone fruit (*Prunus* spp.) and of pecan (*Hicoria* spp.) respectively. The former species, on cherry, is now usually referred to as *Fusicladium cerasi* (Rabh.) Sacc., the perfect stage having been identified as *Venturia cerasi* Aderh. The name *Cladosporium carpophilum* is retained for the strain infecting peach and plum, in which no perfect stage has been recorded. The latter species, *C. effusum*, formerly known as *Fusicladium effusum* Wint., was transferred to the genus *Cladosporium* by Demaree (1928), on account of its catenulate conidia, a character which according to recent (unpublished) observations of d'Oliveira is of doubtful systematic value. Both these species are characterized by the formation of superficial or subcuticular stromata from which the conidiophores are developed, a feature which would tend to suggest closer affinities with *Fusicladium* Bonorden than with *Cladosporium* Lk. The same feature was also used by v. Höhnel (1923) to separate *Fusicladium* from the related genus *Passalora* Fries. A further investigation of the systematic value of these characters is desirable.

VII. Summary

Cladosporium fulvum

1. Symptoms of infection on resistant and susceptible varieties of tomato (*Lycopersicum esculentum* Mill.) are briefly indicated, together with a numerical method of recording the progress of infection.

2. The histology of infection on tomato varieties is described. Penetration is stomatal and no appressoria or other modifications are formed. The mycelium is intercellular and without haustoria, and develops normally only for so long as the host cells are alive.

3. By the use of a controlled environment chamber, the frequency of penetration is shown to be far greater at a humidity fluctuating from saturation to 85 % than at constant saturation. The suggestion is made that penetration is controlled, in part at least, by a hydrotropic stimulus.

4. The initial rate of spread of the mycelium within the host, immediately subsequent to penetration, is lower in the variety "Maincrop" than in "Giant Red" and is lower also in the basal region of the plant than in the middle. This behaviour runs parallel with the subsequent differences observed in the external symptoms of infection.

5. Normal stomatal penetration occurs over a wide range of immune and "inappropriate" hosts.

6. Symptoms of infection have been recorded only in the varieties of *L. esculentum*, in *L. Humboldtii* Dun., and in two strains received as

L. racemigerum which appear to be more closely related to the cultivated varieties than to the true currant tomato.

7. Confusion occurs as to the correct nomenclature of the currant tomato. The name *Lycopersicum pimpinellifolium* Mill. is preferred. *L. racemigerum* Lange (=*Solanum racemigerum*) and *L. racemiforme* Lange are synonyms.

8. The extent and distribution of the mycelium in the currant tomato appears to be determined largely by the age and relative maturity of the leaf. The mycelium is intercellular and without haustoria, and can apparently remain alive in the leaf for a considerable period of time. No conidiophores are produced. No reaction of the host cells is observed except where, on account of structural considerations, host and parasite are brought into more immediate contact. Here, individual host cells are frequently necrotic.

9. On many other Solanaceae, including species of *Solanum*, *Hyoscyamus*, *Nicotiana*, *Schizanthus*, and other genera, the course of events is essentially similar to that observed on the currant tomato.

10. In other Solanaceae, and in plants belonging to the Scrophulariaceae, Compositae, and Cucurbitaceae, an extensive mycelium is never developed, even under apparently favourable conditions.

Cladosporium cucumerinum and *C. herbarum*

11. *C. cucumerinum* is pathogenic to fruits and foliage of cucumber (*Cucumis sativus* L.), also to the young shoots of *Bryonia dioica* Jacq.

12. Both *C. cucumerinum* and *C. herbarum* are capable of penetrating the stomata of a wide range of plants in a manner apparently identical with that observed in *C. fulvum*.

13. *C. herbarum* is not considered potentially parasitic on the plants inoculated.

14. From a consideration of the behaviour of the three species investigated, the suggestion is made that the following features may prove characteristic of the pathogenic behaviour of the genus *Cladosporium* as a whole:

Stomatal penetration by unaltered germ tubes: intercellular mycelium without haustoria: conidiophores subepidermal in origin, not forming superficial or subcuticular layers.

Intercellular mycelium refers typically to infection of foliage. Examples of the application of this hypothesis are given.

VIII. Acknowledgements

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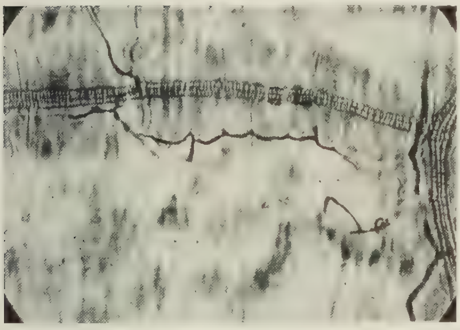
Fig. 1.

Fig. 2.



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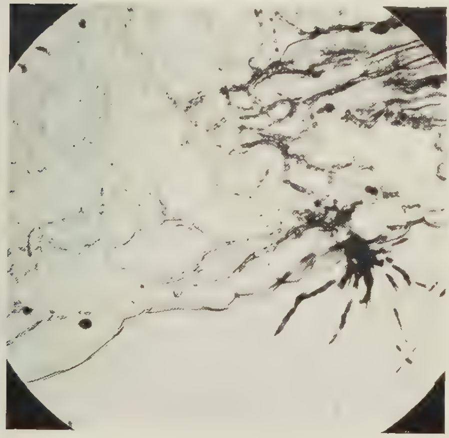
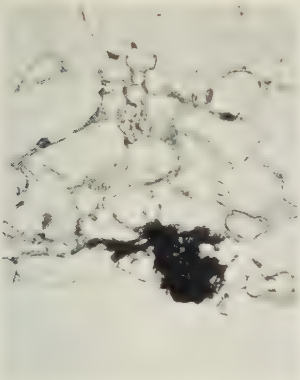


Fig. 5.

Fig. 3.

BOND.—INFECTION EXPERIMENTS WITH *CLADOSPORIUM FULVUM* COOKE AND RELATED SPECIES (pp. 277–307)



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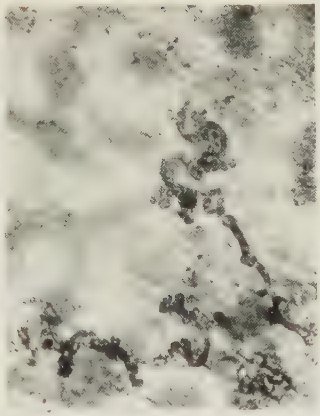

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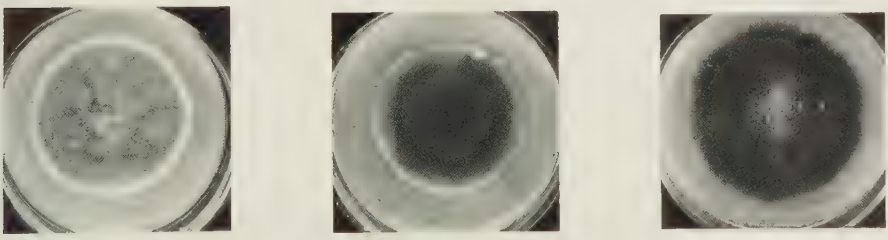

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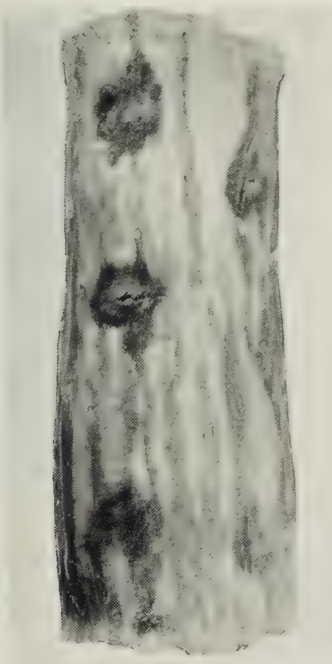

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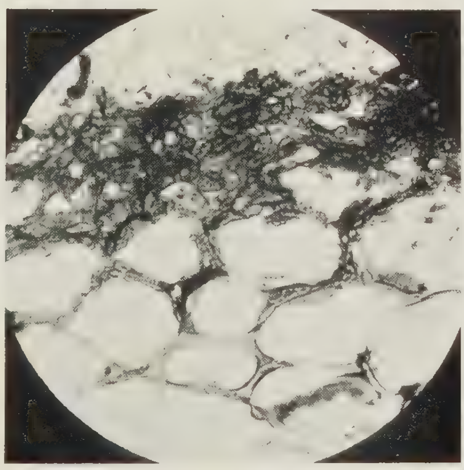

Fig. 5.

BOND.—INFECTION EXPERIMENTS WITH *CLADOSPORIUM FULVUM* COOKE AND RELATED SPECIES
(pp. 277–307)

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EXPLANATION OF PLATES XI AND XII

PLATE XI

Fig. 1. Middle leaflets of "Giant Red" tomato infected 5 weeks previously. (No manure.) $\times \frac{3}{4}$.

Fig. 2. Upper leaflet of "Maincrop" tomato, showing sharply localized lesions, after 5 weeks (0·5 g. sulphate of ammonia weekly). $\times \frac{3}{4}$.

Fig. 3. Middle leaflets of "Maincrop" tomato, as in fig. 1. $\times \frac{3}{4}$.

Fig. 4. Mycelium in "Giant Red" tomato, 4 days after inoculation. Lacto-phenol preparation, showing intercellular hypha following the vascular parenchyma. $\times 250$. (Part of Text-fig. 1.)

Fig. 5. Sporulation in "Giant Red" tomato, showing fertile stroma with intercellular hyphae, after 12 days. Section at $12\,\mu$., stained carbol thionin blue and orange G. $\times 250$.

PLATE XII

Fig. 1. Sterile stroma in "Maincrop" tomato, as in Pl. XI, fig. 5. $\times 250$.

Fig. 2. Distorted hyphae in necrotic spongy mesophyll, after 25 days. Lacto-phenol preparation. $\times 250$.

Fig. 3. Plate colonies on Dox's medium, after 4 weeks at 22·5° C. $\times \frac{2}{5}$. (*a*) *C. cucumerinum*, strain 1/36 (smoke grey, 21′′′′′ O-YY d). (*b*) *C. cucumerinum*, strain 2/35 (citrine drab, 21′′′ O-YY i). (*c*) *C. herbarum* (dark greyish olive, 21′′′′ O-YY k). (Colours from Ridgway's *Colour Standards and Colour Nomenclature*.)

Fig. 4. Part of cucumber fruit infected with *C. cucumerinum*, strain 2/35, after 9 days at 22·5° C. $\times \frac{2}{3}$.

Fig. 5. Section of sporulating lesion above, at $12\,\mu$. $\times 250$.

(The writer is indebted to Mr E. T. Scott for assistance with photography.)

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OBSERVATIONS ON THE SPOTTING OF TOMATO FRUITS BY *BOTRYTIS CINEREA* PERS.

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(With Plates XIII and XIV and 2 Text-figures)

This paper describes a characteristic spotting of both field and glass-house-grown tomato fruit which has been recorded in Great Britain for a number of years (Walton, 1937)[1]. The nature of the causal agent was the subject of much surmise until recently when Read (1937) demonstrated experimentally that this spotting was caused by *Botrytis cinerea*, and suggested the name "water spot" for the disease.

In the past *Botrytis* spotting has been confused with the stigmonose of tomato fruit caused by aphides (see below, p. 319), first recorded in this country by Bewley (1923).

Symptoms

On green unripe fruit a typical *Botrytis* spot consists of a minute brownish puncture at the centre of a pale green or silver-coloured circle varying in diameter from 0·2 to more than 0·5 cm. (the spots are at first small but increase in size as the fruit swells). The area within the circle may be of normal green appearance or paler in colour than the rest of the fruit (Pl. XIII, fig. 1), and there is often a slight swelling around the central spot. There are many variants of this typical spotting. There may be two or more concentric circles (Pl. XIII, figs. 2, 3), two or more adjacent spots may coalesce (Pl. XIII, fig. 2), the spots may be entirely silvery in appearance (Pl. XIII, fig. 1 (left-hand fruit)), or the surrounding circle may be very inconspicuous or even absent as in Pl. XIII, figs. 4, 5, where circular areas, darker green in colour than the rest of the fruit, surround the central punctures. If the silvery rings are absent the spots become less conspicuous as the fruit ripens, but when rings are present they remain prominent on the ripe fruit and appear yellow in colour. The spots usually occur more frequently at the calyx end of the fruit (Pl. XIII, figs. 1, 2), i.e. in the position where floating spores are

[1] Rather similar spots ("bird's-eye" spots) of unknown origin were found associated with *Bacterium vesicatorium* spot of tomato fruit in Indiana by Gardner and Kendrick (*J. agric. Res.*, **21**, 127, 1921 and *Phytopathology*, **13**, 312, 1923).

most likely to lodge, but occasionally severe spotting of the blossom end is seen (Pl. XIII, fig. 4), due to the infection of a persistent dead corolla by *Botrytis* instead of the more usual *Penicillium* sp.

To account for the spotting the following explanation is suggested. Air-borne *Botrytis* spores settle on the surface of the fruit. Under conditions of temporary high humidity these spores germinate, the germ tubes penetrate the epidermis killing the cells around the points of entry but, with the return of less humid conditions, the sporelings are desiccated so that no fungus can subsequently be isolated. The germ tube has, however, secreted pectinase enzyme into the region penetrated and, as this enzyme diffuses outwards, the middle lamella between the epidermis and the underlying cells is destroyed and the ring surrounding the spot appears.

This hypothesis has not been disproved by the experiments, now to be described, designed to test it or to explain the success of the method finally developed for producing the spots experimentally.

METHODS

The experimental work was done during the spring and summer months with tomato plants of the variety E.S. 1 grown in 6-in. pots in a well-ventilated greenhouse. The plants were usually "stopped" after setting two trusses, but, occasionally, a third truss was allowed to set. The truss to be inoculated, trimmed to bear four to seven fruits, the largest being 2·5–3·0 cm. in diameter, was sprayed with a suspension of *Botrytis* spores ("sprayed") or the fruits were dusted with dry spores by means of a camel-hair brush and given no further treatment ("dusted"), or subsequently sprayed with water ("dusted and sprayed"). The sprayed or dusted truss was then introduced into a sterilized wide-mouthed (bolthead) flask containing a little water, the mouth plugged with cotton-wool and the flask held in position by wiring it to the stake supporting the plant (see Pl. XIV, fig. 5). The plant was then well watered. After 16–17 hr. the flask was removed and the truss kept under observation. Spots had frequently appeared by the time the flasks were removed and the fruit was usually re-examined during the next 24 hr. and again at intervals to observe the growth in size of the spots. The inoculations were usually made in the evening and the flasks removed the next morning, and it was customary to keep drops of the spore suspension employed on slides in a moist chamber overnight and to estimate the spore germination the next morning. Experiments confirmed the fact that *Botrytis* spores germinate less readily with age and,

for most experimental inoculations, the spores employed were taken from 7 to 10-day-old cultures on malt or potato agar.

During 1936 the details of this method were being worked out, and its success may be seen by comparing the 1936 and 1937 results which are set out in Table I.

Table I

Summary of experiments with Botrytis cinerea

Method of inoculation	No. of trusses treated	No. of fruit inoculated	No. of fruit spotted	% spotted
1936				
Sprayed	21	76	41	54·0
Controls	6	19	2	10·5
1937				
Sprayed	30	171	147	—
Dusted	9	42	34	—
Dusted and sprayed	6	26	25	—
	45	239	206	86·2
Controls	24	118	7	6·2

For record purposes a fruit was scored as "not spotted" or "spotted", with a note as to the severity of the spotting, and the actual number of spots was not usually counted. *Botrytis*-treated fruit generally developed a large number of spots, while a control fruit, spotted as the result of contamination by air-borne spores, developed only two or three spots (see below, p. 316).

Factors which influence spotting

(1) *Fruit size.* When the 1936 results were rearranged according to the size of fruit inoculated (see Table II) it became evident that increased resistance to *Botrytis* attack accompanied increase in size of the fruit.

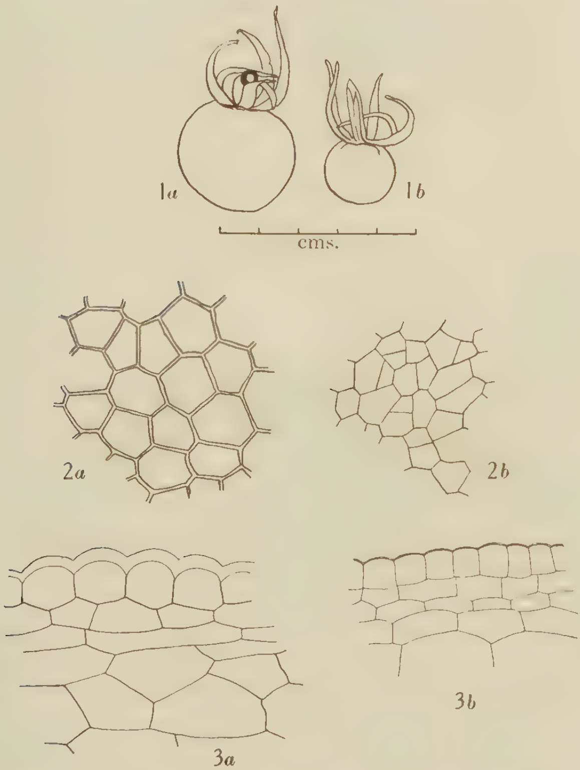
Table II

Effect of size of fruit on spotting

Size of fruit	No. inoculated	No. spotted	% spotted
Large (diam. >3 cm.)	28	1	3·6
Medium (diam. 1·5–3·0 cm.)	16	11	69·0
Small (diam. 0·75–1·5 cm.)	32	29	90·8

Anatomical investigations showed that the increased resistance could be correlated with increased thickness of the epidermal cell walls. Text-fig. 1 shows examples of susceptible and resistant fruits and indicates their anatomical differences (Text-fig. 1 should be compared with

Text-fig. 2, which shows the extremely thickened epidermal layers of a still older fruit). The actual size of a fruit is not always a reliable indication of the fruit's susceptibility to spotting, but increased resistance accompanies the rather sudden change of the fruit surface from a slightly matt to a darker green glossy appearance which occurs during the swelling of a fruit.



Text-fig. 1. Resistant and susceptible tomato fruits. 1, Whole fruit; 2, surface view of epidermis; 3, transverse section of epidermis; *a*, resistant fruit; *b*, susceptible fruit. Detail × 305.

(2) *Turgidity of fruit.* In the early stages of this investigation attempts to inoculate detached fruit kept in a moist chamber met with little success. Comparative trials then showed that fruit on the plant was more easily and more intensely spotted than similar detached fruit when sprayed with the same spore suspension (see Table III, section (*a*), and top line of section (*c*)). It was suspected that this difference in

behaviour could be accounted for by the greater turgidity of fruits still attached to the plant. To test this idea two groups of similar plants were taken and water was withheld from one group until the plants were severely wilting when, after the trusses of both series had been sprayed with the same spore suspension and bottled overnight, normal watering was resumed. It was found that fewer fruits on the wilting plants were spotted and that the spotting was less severe than on turgid plants. The experiment was repeated with similar results, and the two experiments are summarized in Table III, section (*b*).

Table III

The effect of fruit turgidity on spotting

	No. fruit inoculated	No. spotted	% spotted	
Attached	27	13	48·3	*a*
Detached	15	3	20·0	
Plant wilting	51	18	35·3	*b*
Plant turgid	33	27	82·0	
Detached (blossom-end in water)	39	4	10·2	*c*
Detached (calyx-end in water)	41	40	97·5	

Sections *a*, *b* and *c* in the above table are the totals derived from comparable pairs of experiments.

Further trials were made with detached fruit in a moist chamber comparing the effects of placing the blossom-end and the calyx-end (with or without removing the calyx) in water, when it was found that if the calyx-end dipped in water severe spotting occurred. Fruits left overnight with their calyx-ends in water became very turgid, and on account of this great turgidity water-filled blisters developed instead of typical spots. Uninoculated control fruits did not develop blisters, but medium-sized fruits often split.

A possible explanation of the decreased susceptibility of fruit not fully turgid is that, as the penetration of the fungus is purely mechanical and the epidermis slightly elastic, penetration is more easily effected when the epidermis is tightly stretched.

(3) *Variety of tomato*. From general observations some evidence accumulated to show that certain varieties are more susceptible than others to *Botrytis* spot and, though varietal resistance is a factor of minor importance and without practical significance, it was thought worth while to record the following details.

Counts were made of the numbers of plants carrying naturally spotted fruit in four consecutive rows, two of the variety Ideal and two of a Stirling Castle hybrid resistant to leaf mould (*Cladosporium fulvum*), in

one of the experimental houses on the Station nursery. It was found that thirteen of twenty Ideal plants and six of twenty Stirling Castle carried spotted fruit and, in the adjacent house where the experiment was duplicated, the corresponding figures were seventeen of twenty and five of twenty, making the totals thirty plants with some fruit spotted out of forty for Ideal and eleven out of forty for the Stirling Castle hybrid. Also, the Ideal fruits were more heavily spotted than those of Stirling Castle. This suggested a difference of susceptibility between the two varieties and additional evidence was obtained from the results of the experimental inoculations which are summarized in Table IV.

Table IV

Varietal susceptibility

Variety	No. fruit inoculated	No. spotted	No. spots per fruit
Ideal	15	10	20·4*
Stirling Castle hybrid	14	10	11·4*

* The difference between these numbers is not statistically significant.

In another experiment fruits on similar plants of the varieties Ailsa Craig, E.S. 1, Radio and Potentate were inoculated at the same time under the same conditions when, as judged by the eye, there was no difference between the degree of spotting on E.S. 1 and Ailsa Craig, but Radio was less severely and Potentate more severely affected than the first two varieties.

This difference in susceptibility is probably related to differences in the resistance of the epidermal layers but, although fruit of the Stirling Castle hybrid and Radio are seen to have thicker fruit walls than Ideal and Potentate respectively when cut open longitudinally, no definite differences in the structure of the epidermis was observed in freehand sections cut from similarly sized fruits.

(4) *Humidity.* Several experiments were performed to study the degree of humidity necessary to allow spore germination to take place and for spotting of the fruit to result. In the first experiment six plants, each bearing one truss, were taken and the fruit dusted with dry spores. One of the trusses was then enclosed in a flask containing distilled water in the usual manner while, in the flasks enclosing the other trusses, the water was replaced by sulphuric acid diluted to give relative humidities of 90, 80, 70, 60 and 50 %. When the flasks were removed the fruit enclosed over water (i.e. subjected to a R.H. of 100 %) was covered with a thin film of moisture and spots developed on these fruits, while the

surface of all the other fruit was dry and no spots developed. In subsequent experiments fruit was lightly sprayed with a spore suspension and then enclosed in atmospheres of varying relative humidity when, overnight, spotting occurred if the R.H. was 90 % or higher. In one series kept under constant observation the spots of water completely dried off the fruits kept at 80–60 % R.H. in about 5 hr., i.e. dried before penetration of the fruit had occurred.

(5) *Length of time necessary for infection.* To determine the length of time necessary for infection trusses on seven to ten similar plants were sprayed with a spore suspension, enclosed in flasks, and then after varying intervals of time a plant was taken at random, the flask removed and, if necessary, the evaporation of the drops of water remaining on the fruit encouraged with an electric fan. The time from the beginning of the experiment to the disappearance of the drops of water on the fruit was noted. When drops of water remained on the fruit less than 4 hr. no spotting resulted; if they persisted 4–5 hr. a few spots developed, and if 6 hr. or longer the fruit was more heavily spotted. This result was obtained with an average temperature of 18·9° C. (66° F.) during the first 4 hr. of the experiments and when the spores used germinated in water on a slide after 2–3 hr. The germination of the spores in distilled water is only a rough guide as to their behaviour on the fruit. In one of the experiments drops were removed from the fruit and examined after 4 hr. when the length of an occasional germ tube was six times that of the spore, whereas on the slide the length was less than twice that of the spore.

Occasionally, if a large drop of water persisted 18 hr. or longer a soft rot of the fruit set in and this was more marked if the spore suspension was made in a nutrient solution. In other cases, however, when fruit was incubated for periods up to 48 hr. no soft rot resulted, which rather suggests that unripe fruits show a natural resistance to the development of *Botrytis* after penetration. This point was not investigated, but it is possible that this internal resistance is also related to the turgidity of the fruit (cf. the work of Brown (1934) and his collaborators).

(6) *Length of time spores lie on the fruit.* An experiment was carried out to determine how long a spore might lie on a fruit and retain its ability to cause a spot when conditions favourable for germination occurred. One morning seven trusses were sprayed with a spore suspension, the drops of which dried off the fruit within an hour. At intervals one of the plants was taken at random and the sprayed truss enclosed in a flask overnight. The results of this experiment are set out in Table V.

Table V

Length of time spores lie on fruit before "incubation"

When "incubated"	No. fruit	No. spotted	% spotted
Same day	6	6	100·0
After 1 day	6	5	83·5
,, 2 days	10	10	100·0
,, 4 ,,	7	5	71·5
,, 6 ,,	8	4	50·0
,, 7 ,,	3	1	33·3

The plants were carefully inspected each day and no fruit developed spots before being "incubated", and the seventh unincubated truss was unspotted on the eighth day. After 4 days the percentage of spotted fruit declined, and it is interesting to note that it was the largest fruits on the trusses incubated on the fourth to seventh days that failed to spot. It is clear, therefore, that a spore may remain on a suitable fruit for at least a week and retain its ability to cause a spot, and that, although *Botrytis* spores are viable for at least 6 months (Wilson, 1937), it is the growth of the tomato fruit which determines the period of susceptibility.

(7) *Effect of fungicides on spotting.* Three plants were well sprayed with Bouisol and oil at the strength used against leaf mould, and the next day the trusses on these plants and on three similar untreated plants were sprayed with a spore suspension and incubated. Spots developed on both sprayed and unsprayed fruit, but the spots were fewer on the fruits previously treated with the colloidal copper and oil spray.

TESTS TO DETERMINE THE ABILITY OF *BOTRYTIS* SPECIES AND OTHER FUNGI TO SPOT TOMATO FRUIT

Strains of *Botrytis cinerea* Pers. isolated from tomato fruit and stems, *Anemone*, *Statice*, *Zinnia* and fig, caused equally vigorous spotting and, in most of the experimental work, the strain isolated from tomato fruit was used.

Botrytis Tulipae (Lib.) Lind., *B. Allii* Munn and *B. Fabae* Sard. all proved able to cause similar spots to those produced by *B. cinerea* in spite of the fact that, of these four species, only *B. cinerea* caused a soft rot of green tomato fruits when introduced into a wound. *B. Fabae* and *B. Tulipae* gave rise to particularly large spots (Pl. XIV, fig. 1), and this is probably related to the fact that the spores of these species are larger

than those of *B. cinerea*. The results of experiments with *B. Paeoniae* Oud. were inconclusive.

Penicillium sp., isolated from tomato fruit, failed to cause spotting.

Phytophthora infestans (Mont.) de Bary. Field-grown tomatoes attacked by blight and also showing *Botrytis* spots were examined but, under experimental conditions, conidia of *P. infestans* gave rise to a superficial or more general rot but no spots. It is interesting to note that strains of *P. infestans* obtained on potato from Kirton, Lincolnshire and Scotland vigorously attacked potato plants but failed to cause any damage to tomato fruit or foliage, while a strain attacking field-grown tomatoes in Jersey caused a vigorous rot of all the tomato plants and fruit inoculated.

Phytophthora parasitica Dast. Zoospores produced no spots but a general rot (buck-eye rot).

Cladosporium fulvum Cooke. Particular interest is attached to this fungus because of its ubiquitous occurrence on tomatoes in this country, and Read (1937) was originally of the opinion that *C. fulvum* could cause a type of spotting, but this view was not confirmed by the results of the later experiments which are summarized in Table VI.

Table VI

Experiments with Cladosporium fulvum

Fungus	No. fruits inoculated	No. spotted	No. of spots per fruit
C. fulvum	133	20	1·5
B. cinerea	56	49	Many
Controls	108	7	1·3

Unfortunately, it was during certain of the experiments with *Cladosporium* that the greenhouse became contaminated with *Botrytis* spores derived from a neglected *Pelargonium* plant. Eighteen of the spotted fruit in the *Cladosporium* series and the seven in the control series all occurred in four experiments carried out during one week, and evidence that these spots were the result of contamination was afforded by the facts that the number of spots per spotted fruit was very few (see Table VI), the spots were all on the calyx-end of the fruit, a few untreated fruits developed spots at that time, and the trouble ceased with the removal of the diseased *Pelargonium* mentioned above. Of eight further independent experiments, seven gave negative results although in some the incubation period was extended to 24 hr. (longer than the time necessary to initiate leaf infection by *Cladosporium*), and the plants

were kept under observation for a fortnight. In one experiment two fruits of nine inoculated each developed a few typical *Botrytis* spots.

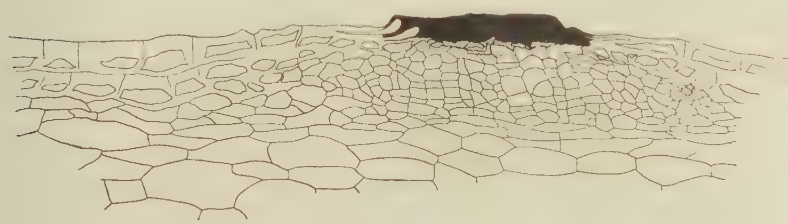
In a few supplementary experiments with detached fruit standing in water, spots or blisters developed on fruit sprayed with *B. cinerea* spores when none occurred on the fruit inoculated with *Cladosporium fulvum*.

The *Cladosporium* spores used were taken from carefully selected young natural infections and, though contamination with *Botrytis* was possible, it was thought to be very unlikely.

It was concluded from this study that *Cladosporium fulvum* does not cause a spotting of tomato fruit and, from *a priori* considerations, if it did fruit spotting should be of more frequent occurrence.

PATHOLOGICAL HISTOLOGY

Penetration of the fungus was observed by mounting epidermal strips in lactophenol and staining with cotton blue and by double staining with thionin and orange G (Stoughton's method) microtome sections cut at 10–20μ. of material fixed in weak Flemming's solution.



Text-fig. 2. Transverse section through the centre of a spot 35 days after inoculation. ×172.

The germ tube develops a penetration peg and enters a single epidermal cell which collapses, and the contents of the adjacent cells become granular. This group of cells is killed, turns brown (Pl. XIV, fig. 2) and so causes the central puncture. It was noticed that when the spores were dusted on to the fruit the germ tubes were much shorter than when the spores were sprayed. In the latter case the germ tubes usually grew to a length of five or more times that of the spore before penetrating the epidermis (Pl. XIV, fig. 2) while the germ tubes from dusted spores were short and penetrated almost immediately, possibly because of their closer contact with the epidermis. In experimental infections, when the number of spores applied was large, single lesions caused by the penetration of several germ tubes were often observed.

The penetration wound is superficial and, usually, not more than two layers of cells below the epidermis are involved. The pale ring surrounding the puncture is caused by a layer of air between the epidermis and the underlying tissue, but, in stained preparations, the epidermal cells surrounding the central puncture have appeared normal.

Sections were also cut of spots fixed at varying intervals up to 35 days after inoculation. No trace of mycelium was detected in material fixed 4 days after inoculation when a few cells below the site of penetration were seen to be dividing and, later, a well-developed layer of wound tissue is laid down (see Text-fig. 2). It is this wound tissue which causes the swelling often seen in the centre of an old spot.

Experiments with enzymes

Attempts were made to produce spots by means of pectinase enzyme prepared from the germ tubes of *Botrytis cinerea* by Brown's method (1915). After testing the activity of an enzyme preparation, by observing its ability to disintegrate thin slices of carrot and potato tuber, drops of the enzyme were placed on the surface of tomato fruits both on the plant and detached and either in a moist chamber or not. The surface of the fruit beneath the drops was then pricked with a very fine insect pin fitted with a cork shield so that the depth of the puncture was not more than about 0·2 mm., or a larger wound was made with a dissecting needle. It was found that drops of enzyme placed on the surface of the fruit without injury to the underlying epidermis caused no damage even when they persisted for more than 24 hr. If the enzyme was boiled for a few minutes before applying it to the fruit the puncture below a drop differed in no way from a control puncture through a drop of distilled water but, when the puncture was through untreated enzyme, the surrounding tissue turned brown within 12 hr. and a small brown spot similar to, but larger than, that at the centre of a natural infection resulted whether the fruit was enclosed in a moist chamber or not (Pl. XIV, fig. 3). Occasionally, a very faint ring developed around the puncture but, as soon as the green fruit began to ripen, the spot became prominent because a circle of tissue around the puncture ripened more slowly (Pl. XIV, fig. 4).

As can be seen from the figures, the resemblance of spots produced by the enzyme to natural spots is not very close but the difference may perhaps be accounted for by the manner in which the enzyme was introduced. The ring which results from a natural infection is undoubtedly a

diffusion phenomen but no explanation of the exact mechanism of ring formation can be offered.

The only difference between enzyme spots on attached and detached fruits was that as an attached fruit increased in size the centre of the spot became slightly depressed.

Enzyme prepared from *B. Fabae* differed in no way from the *B. cinerea* enzyme and caused similar effects on the fruit. This suggests that the larger sized spots associated with *B. Fabae* are due to the larger amount of enzyme introduced.

EXPERIMENTS WITH SUNLIGHT

One explanation of the origin of *Botrytis* spots was that sunlight focused on to the surface of the fruit by means of water droplets damaged the epidermis but experiments to test this explanation have given negative results. At different times a total of thirty-three fruits on seven trusses were exposed near the glass to direct bright sunlight during June and July. The plants were sprinkled with tap water and, when this dried, sprinkled again, but no damage resulted.

Walton (1937) was of the opinion that sunlight caused the spotting, but his experimental evidence is open to an alternative explanation, since the repeated damping over of the plants would make conditions very suitable for *Botrytis* attack to occur. In this connexion it may be noted that natural spotting frequently occurs on lower trusses never exposed to direct sunlight, but subject to very humid conditions, and that although strong sunlight is able to scorch the leaves of soft plants, and is a contributary cause to "green-back" of fruit, no evidence has been obtained that it damages the fruit in any other way.

STIGMONOSE

For several years we considered insects to be the cause of *Botrytis* spot, but Read (1937) has shown experimentally that the aphid *Myzus convolvuli* Kalt. (= *M. pseudosolani* Theob.) is able to damage tomato fruit and that this damage differs from *Botrytis* injury (see Pl. XIII, fig. 6 and compare with figs. 1–5). It was proposed that the name stigmonose, first applied by Bewley (1923) to aphid damage of tomato, should be retained for aphid injury which occurs naturally but infrequently. The spots caused by aphides are pale in colour, slightly raised with margins less clearly defined than those of spots caused by *Botrytis*, and with, or without, a very minute dark central puncture. Examination of the fruit usually shows traces of aphides (Pl. XIII, fig. 6). Cast skins

may remain attached to the fruit, particularly around the calyx which often shows a yellow mottle.

Control

It will be clear from the above results that, for *Botrytis* spotting to occur, *Botrytis* spores and excessive humidity are necessary. The source of *Botrytis* spores is usually infected snags and plant debris resulting from careless pruning and, under glass, the excessive humidity, which also encourages *Botrytis* rot, may be caused by careless watering and ventilation. *Botrytis* spotting usually reflects faulty cultural methods and its control is, therefore, largely a matter of employing the best horticultural practice. Stigmonose can, if necessary, be controlled by fumigation.

Summary

Characteristic ring-like spots caused by *Botrytis cinerea* on tomato fruits are described, with observations on certain factors which influence the spotting.

Evidence is presented to show that, under conditions of high humidity, *Botrytis* spores lying on the surface of immature fruits germinate, penetrating the epidermis, and that the spots result from the pectinase enzyme secreted by the germ tubes. With the return of drier conditions the sporelings die and no fungus can be isolated from mature spots.

It was found that similar spots could be produced experimentally by other species of *Botrytis* but not with various other fungi able to attack tomatoes.

The *Botrytis* spots are compared with aphid injury and notes are given on control.

We wish to thank Mr G. Cockerham, Mr E. R. Wallace, and Dr T. Small for material of blight, and Mr W. C. Moore for records of the disease.

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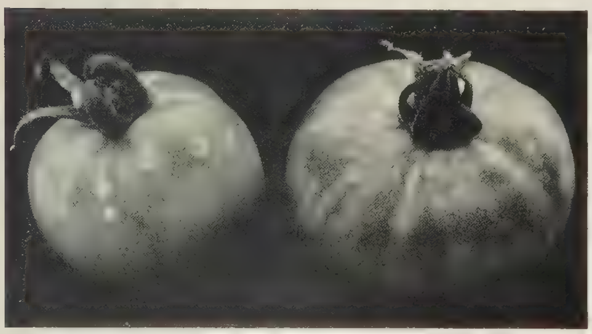

Fig. 1.

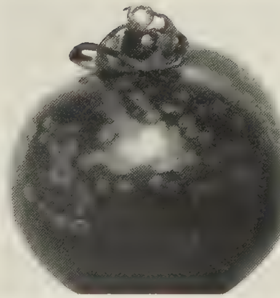

Fig. 2.

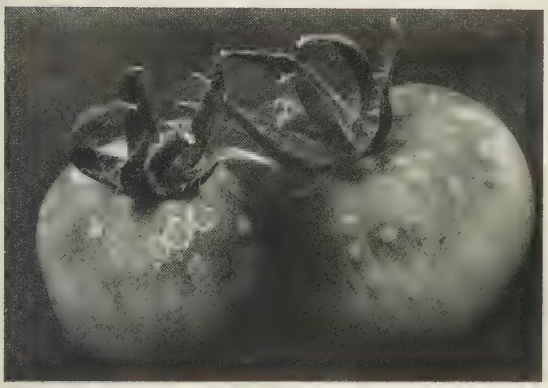

Fig. 3.

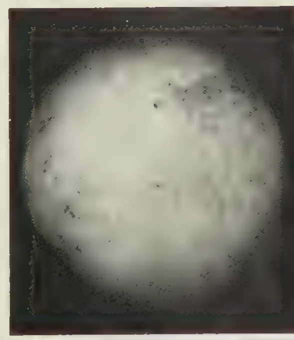

Fig. 4.

Fig. 6.

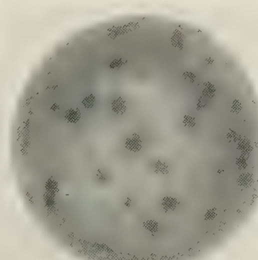

Fig. 5.

AINSWORTH, OYLER AND READ.—OBSERVATIONS ON THE SPOTTING OF TOMATO FRUITS BY *BOTRYTIS CINEREA* PERS. (pp. 308–321)

Fig. 1.

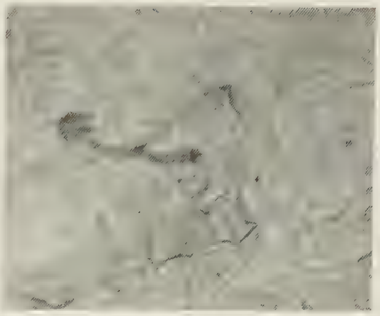

Fig. 2.

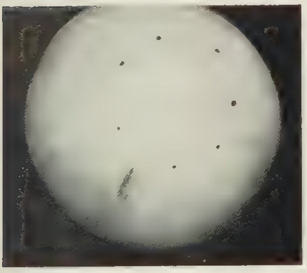

Fig. 3.

Fig. 4.

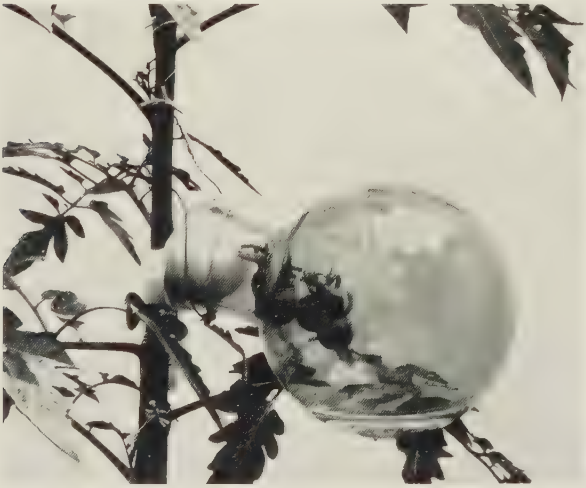

Fig. 5.

AINSWORTH, OYLER AND READ.—OBSERVATIONS ON THE SPOTTING OF TOMATO FRUITS BY *BOTRYTIS CINEREA* PERS. (pp. 308–321)

EXPLANATION OF PLATES XIII AND XIV

PLATE XIII

Fig. 1. Natural infection, unripe fruit.
Fig. 2. Natural infection, ripe fruit.
Fig. 3. Experimental infection with *B. cinerea*, unripe fruit.
Fig. 4. Natural infection of blossom-end, unripe fruit.
Fig. 5. Experimental infection with *B. cinerea*.
Fig. 6. Stigmonose caused experimentally by *Myzus convolvuli* Kalt.

PLATE XIV

Fig. 1. Experimental infection with *B. Tulipae*.
Fig. 2. Germ tube penetrating epidermis, 16 hr. after inoculation. (Temporary mount in lactophenol stained with cotton blue.) $\times 460$.
Fig. 3. Spots resulting from punctures with an insect pin through drops of enzyme. Control punctures alternate with the spots.
Fig. 4. Enzyme spots and control punctures on half-ripe fruit. (The punctures were made with a dissecting needle.)
Fig. 5. Method used for "incubating" experimental infections.

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EXPERIMENTAL SPAWN AND MUSHROOM CULTURE

II. ARTIFICIAL COMPOSTS

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(With Plates XV and XVI)

CONTENTS

Introduction

The experimental results of preliminary tests for the growth of different species of *Psalliota* on artificial composts have been described (Cayley, 1937), but the details of the proportions of the ingredients and the methods of composting were deferred to a later publication. In the previous paper, the records of tests with three wild species and several varieties of cultivated mushrooms on well-composted stable manure showed definitely that the wild species *Ps. campestris*, the wild grassland species generally known as *Ps. arvensis*, and the wild haystack mushroom (sp. undetermined) would not grow on stable manure compost. Experiments with artificial composts were then started with the object of finding some suitable medium for the growth of the wild species.

The main object of this paper is to supply details as to ingredients and methods of treating artificial composts, to describe further more extensive experiments, and to discuss the suitability of the various composts for the growth of the cultivated varieties of mushroom.

It must be pointed out however, that over and above laboratory

tests in large tubes and battery jars, the composts to be described below have, for the most part, only been tested in large 12 in. pots and a few larger boxes, owing to lack of space and adequate accommodation. The results, however, show that previous high temperature fermentation of the compost is not essential for the growth of the mushroom itself, although in the case of the saprophytic cultivated varieties, previous fermentation may make the necessary nutritive ingredients more easily available to the fungus. On the other hand, when dealing with manure or any other material containing a high percentage of plant residues, composting is essential in order to reduce the compost to a suitable physical condition for mushroom growth and to prevent excessive heating and burning. Where artificial heat is not available, the heat generated during composting and retained in the bed is of importance.

American workers (Waksman & Nissen, 1931; Waksman & McGrath, 1931) and others (Falck, 1928) investigated the chemical changes which take place in manure during fermentation and after the growth of the mushroom, and found that during the process of high-temperature composting of stable manure there is a considerable increase in the relative lignin content of the heap, and that after the growth of the mushroom this lignin content was greatly decreased. The American investigators concluded that the mushroom, in some way, utilized a considerable amount of lignin, more especially as a similar depletion of lignin occurred in sterilized stable manure and, hence, could not be attributed to the agency of other micro-organisms. They also held that "the need for the composting of manure, in order to develop a favourable medium for the growth of mushrooms, consists in bringing about an enrichment in the lignin and protein content and possibly a change in their chemical nature" (Waksman & Nissen, 1932, p. 272). They considered, also, that lignin and protein are the most essential nutrients for the mushroom. Falck (1928) also observed the same increase in lignin content brought about by high temperature fermentation. These points will be discussed more fully later.

INGREDIENTS FOR ARTIFICIAL COMPOSTS

A number of different artificial composts for manure and mushroom growing have been devised by various investigators with variable results which cannot be considered in detail here (Demelon & Burgevin, 1932, 1935; Demelon *et al.* 1935; Hébert, 1911; Hein, 1930 *a*, *b*; Hutchinson & Richards, 1921–3; Lambert, 1929; Paterson-Hart, 1936; Waksman & Reneger, 1934).

In view of the fact that, up to the present time, stable manure has been almost exclusively used for mushroom beds by the trade and private growers, the ingredients for the artificial composts described in this paper were based on the food of horses, namely, hay, grass and crushed oats, with the addition of straw. These ingredients are easily obtainable in this country and at a low cost.

Proteins and a complete plant food are supplied by the hay and grass, and a liberal addition to the lignin supply is introduced by the straw. The crushed oats were only used in the standard medium for spawn growing (Cayley, 1937) and not in the composts prepared in bulk.

Fermented high-temperature composts

The first series of experiments were made with fermented and composted straw and hay treated as for stable manure. In bulk the composts generated a considerable amount of heat only little short of that of fermenting stable manure, but it was found that the artificial composts did not retain the heat for so long a period. This may be due to physical conditions, but the capacity for heat retention has not been investigated.

The treatment of one compost during fermentation is set out in some detail below as a standard method of composting. All the other fermented composts were treated in much the same way, but the temperature of some rose higher than others and the composts had to be turned at shorter intervals.

Artificial Compost I

Ingredients:

Two trusses of wheat straw.
Two trusses of hay (2 years old).
One large sack of chopped straw.
One large sack of chopped hay.
1 lb. sulphate of ammonia.
4 lb. slaked lime.
½ lb. phosphate of potash.

Stack in thin layers in the following order: long straw, chopped hay, long hay, chopped straw, damping each layer thoroughly with a fine rose can.

Stack to about 3 ft. and keep the surface of the stack moist by sprinkling with a fine rose can.

Leave stacked till the temperature rises to 110–120° F.

First turning. Turn over and shake to loosen any dry lumps of hay, taking care to place the outer layers in the centre of the stack. Water during this process with a solution of 2 oz. sulphate of ammonia to the gallon of water until 1 lb. has been added

in all. More water may be added if necessary, as the compost at this turning must be thoroughly moistened but not made too wet.

Stack to 3 ft. and leave till the temperature has reached its maximum (130–140° F.) and has begun to fall.

Second turning. Turn again in the same way. The compost at this stage may smell rather rank and present a very mouldy appearance. Stack and leave till the temperature begins to fall.

Third turning. Turn again and add 4 lb. slaked lime and ½ lb. phosphate of potash in powder form, distributing it as evenly as possible throughout the compost, and stack again. The rank smell will disappear after the addition of lime and the temperature will rise rapidly to about 150–155° F.

Fourth turning. Turn when the temperature has begun to fall and make up the permanent bed at this turning. Heap the compost to about 6 in. above the required level to allow for settling. Press down the compost gently but not too firmly. This is best done with a potato fork. After settling, the bed should not be more than 8 in. in depth. Press again about once every 2 days till the compost is sufficiently firm. Keep a thermometer plunged in the centre of the bed. The risk at this stage is that the bed may dry out or heat in the middle. If the temperature drops unduly fast, this indicates that the bed is too dry in the centre and must be lightly forked up, a little water added and remade. The temperature at this stage should drop gradually. Spawn at 70–75° F.

If dry spawn is used it should be moistened before planting; this reduces the incubation period between spawning and cropping by about 3 weeks.

After 3–4 weeks (or longer) the bed can be cased with soil containing 5% lime to a depth of 1½ in. Should the compost be rather on the wet side casing should be deferred till the surface has dried somewhat.

The moisture content of the compost when the bed is made up, is of the utmost importance; it should be felt in the hand at each turning and water added with discretion. A fine rose can should be used throughout.

This compost is more suitable for small rather than large beds. Ridge beds were not tried. In bulk it was not satisfactory when tested under cover in a cold cellar. It was not sufficiently broken down and friable and settled too firmly. A bed 14 sq. ft. and 10 in. deep was tested with bought commercial spawn; but unfortunately the spawn was of poor quality; the crop on the control bed was much below the average and only a few medium-sized pilei developed on the compost bed.

The temperature registered in the compost bed 1 month after spawning was 60° F.

In smaller bulk, however, in a wooden box $2 \times 1\frac{1}{2}$ ft., spawned with the same but a different sample of commercial spawn at 70° F. and kept under warmer conditions in a greenhouse, produced a good flush after 10 weeks and a second (Pl. XV, fig. 9) some 14 days later. The box was then discarded. This box was watered with ½ oz. phosphate of potash to the gallon of water after spawning.

This result suggested that the physical conditions, such as moisture content, and aeration and that the general conditions for growth in the larger bed were not satisfactory.

The two wild grassland species and the wild haystack mushroom were also tested in smaller beds in the cellar but failed to make any growth.

In the open this compost gave better results. Working in collaboration with the author, a mushroom bed of the same ingredients and treated in the same way, was made up in the open in November by Miss F. M. Durham at Otterton, Devonshire. The compost was turned four times and reached a temperature of 150° F. The bed was spawned at 70° F. with oatmeal-agar cultures of two cultivated varieties, and protected against winter weather conditions with straw, sacking and corrugated iron. Some 5–6 months after spawning, this bed produced a fair crop of several flushes.

A second compost was treated in bulk.

Artificial Compost II

Ingredients:

Two sacks chopped straw.
Two sacks chopped hay.
6 oz. sulphate of ammonia.
2 lb. slaked lime.
$\frac{1}{2}$ lb. phosphate of potash.

Turned four times during the course of 9 days on account of the rapid rise of temperature and reached a maximum of 165° F. on the eighth day. On the ninth day it was divided and made up into four small beds in a wooden frame with glass partitions. On the tenth day the temperature dropped rapidly and it was found that the compost was too dry. It was forked up, 1 oz. of lime and a 2 gall. pail full of freshly cut lawn mowings added to each bed and watered. This treatment raised the temperature, which varied in the four beds from 78 to 95° F. and the *p*H from 7·95 to 8·1.

Two beds were spawned with oatmeal-agar cultures of two cultivated varieties (at 72 and 82° F.), a third with an oatmeal-agar culture of the wild haystack mushroom (at 81° F.), and the fourth left unspawned as control.

This compost also gave very unsatisfactory results, but nevertheless one of the cultivated varieties produced a good clump of normal pilei 4 months after spawning.

These preliminary tests in bulk confirmed the tests made in large tubes and battery jars under more controlled conditions and showed that the cultivated varieties could develop normal pilei on a fermented medium other than stable manure, consisting of straw and hay.

The wild haystack mushroom which has been shown to be intermediate in its physiological properties between the two wild grassland species and the cultivated varieties (Cayley, 1937) failed to grow in beds of Composts I and II. In battery jar tests however, under more strictly controlled conditions, it was found to tolerate Compost II if sterilized on the fourteenth day and further fermentation arrested. The wild grassland species were not tested on this compost.

Compost I was repeated the following year and again tested in the cold cellar. This time the phosphate of potash was omitted and only 12 oz. of sulphate of ammonia added. Half the compost was treated with lime and the other half left without; it was turned four times and placed in the permanent bed at the fourth turning. The maximum temperature attained with lime was 130° F. (pH 8·08) and that without lime 124° F. (pH 8·49).

In general, the addition of lime to a compost fermented at a high temperature produces little or no effect on the pH. As can be seen in Table I, in composts without soil it has not increased the alkalinity above that of the same compost without lime, except in Division II by 0·02, and Division III by 0·03. In unfermented composts, on the other hand, the addition of lime does affect the pH, but not to any marked degree.

Beds of this compost were tried in the cold cellar, and spawned in November with proper spawn of a cultivated variety $9\frac{1}{2}$ months old. The low temperature of the cellar was not conducive to rapid growth. The following May numerous incipient fruiting bodies appeared on both beds but few reached maturity. After 9 months the beds were forked up and it was found that the compost had settled too firmly and that aeration was totally inadequate. In both beds, with and without lime, the compost was sticky and the spawn had not spread evenly through the beds but occurred in patches and was stringy. Apart from being sticky and too firm, the compost had no rank smell and the water content appeared to be satisfactory. In large pots or boxes on the same composts, cultures of the cultivated varieties, spawned with spawn 8 months 10 days old, produced normal pilei (Table I, Composts III, IV, Pl. XV, figs. 2, 10).

These results again suggested that owing to the sticky condition of these composts in bulk, the main cause of failure was lack of adequate aeration.

Low-temperature composts

The low-temperature composts mentioned in the previous paper (Cayley, 1937) were made with the same ingredients as Compost II.

Ingredients:

1 lb. chopped straw.

1 lb. chopped hay.

Mixed and moistened with 2 oz. sulphate of ammonia to the gallon of water, packed in a large biscuit tin and allowed to ferment. After 10 days ½ oz. slaked lime mixed in and the compost replaced in the tin. Maximum temperature attained 90° F.

Samples were taken out and sterilized at intervals and different periods of fermentation tested in battery jars and large tubes.

The wild grassland species failed to grow in any low-temperature compost; the wild haystack mushroom grew and fructified on a compost subjected to fermentation for 1 month but not longer, and the cultivated varieties grew well on composts subjected to fermentation of 1–2 months' duration.

Composts naturally rotted without heat

A further more comprehensive series of experiments were then made both with fermented straw, hay and dried lawn mowings, and the same ingredients allowed to rot naturally without heat in the open. For the different mixtures of these ingredients see Tables I and II.

Although it is possible that some mild form of fermentation takes place without the generation of heat in damp plant residues in the open, in this case, it was of short duration (3–4 weeks), and only a slight degree of disintegration took place before spawning. It is essential, if dried lawn mowings are used, that they should be freshly cut and thoroughly dried. They should be spread out in thin layers protected from rain and not allowed to generate any heat. If used undried they make the compost too moist and soapy.

Method of preparing naturally rotted composts

Mix the ingredients thoroughly, moisten with a fine rose can and spread out in a thin layer on a concrete or paved surface. The surface of the compost is then watered with a solution of 2 oz. sulphate of ammonia to the gallon of water, but not so heavily that the solution drains through rapidly. If the quantity of compost is small a more concentrated solution can be used. The mixture is left spread out for 10–11 days and the surface kept moist during hot sunny weather. After this interval the mixture is divided in half, slaked lime sprinkled over one half and the other half left without. Both halves should be lightly watered and left spread out for another 10 days or longer. The compost can then be made up into beds and spawned without risk of heating provided it is not used in too great bulk. An equal volume of soil is added to those composts with soil when the beds are being made up.

COMPOST TESTS WITH CULTIVATED VARIETIES

The majority of the later tests, both with fermented and naturally rotted composts, were made in large 12 in. pots and a few boxes $2 \times 1\frac{1}{2}$ ft. The pots were spawned as soon as the composts had been prepared, a period extending over some weeks from September onwards. The pots and boxes were kept in a wooden shed during the winter, covered with blanketing and a paraffin stove used to keep out the frost. The temperature of the shed during the winter months ranged from 33 to 56° F., conditions far from ideal for mushroom growing.

As controls, some of the unfermented composts were sterilized in glass battery jars and the cultures kept under aseptic conditions in the laboratory. These controls gave interesting results, as the development and spread of the mycelium in the different composts could be kept under observation. These will be dealt with later.

The results of the tests with cultivated varieties in pots on twenty-four different composts are set out in Tables I and II. It can be seen that nineteen out of the twenty-four tests were positive; of the remaining five the negative results were with Composts XI XIII, Table I, Division III, might possibly be due partly to the toxic effect of oxalic acid and partly to unsuitable physical conditions, and the two tests, Composts VII and VIII, were made with single-spore cultures. Hence the cultivated mushroom will crop on a fairly wide range of media both fermented and naturally rotted without heat, with and without lime and soil, but the most successful results were obtained with fermented and naturally rotted composts containing both soil and lime. All the various composts proved to be on the alkaline side.

In Table I, Divisions I and II, and in Table II, Division IV, 5 g. commercial (neutral) superphosphate of lime was added per pot and watered in after spawning. In Table II, Divisions V and VI, no superphosphate was added, as all the composts showed a pH of less than 8. Fermented mixtures containing dried lawn mowings, Table I, Division III, are not to be recommended, as the resulting product is a lumpy, sticky, highly alkaline mass.

As these composts (Division III) were so alkaline and the physical condition so unsuitable, 0·5 l. of 0·01 % oxalic acid was added per pot, partly to reduce the alkalinity and possibly alter the physical condition, and partly with the object of finding out whether oxalic acid is toxic to the mushroom. The mycelium of all the forms of mushrooms under investigation is thickly encrusted with crystals of calcium oxalate when

Table I

Fermented artificial composts. High-temperature composting. Pot cultures spawned with cultivated varieties

Division I. Composts III–VI.

One large sack chopped straw } + 6 oz. sulphate of ammonia.
One large sack chopped hay }
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
17 days composting. Max. temperature 150° F.

Compost ...	III +l. −s.	IV −l. −s.	V +l. +s.	VI −l. +s.	
pH	8·32	8·39	7·58	7·51	5 g. superphosphate of lime added per pot, and watered in after spawning
Origin of culture	Stipe	Stipe	Stipe	Stipe	
Fructification	+ +	+ +	+ +	+ +	
	Pl. XV, fig. 10	Pl. XV, fig. 2	Pl. XV, figs. 3–5	Pl. XV, figs. 6, 7	

Division II. Composts VII–X.

One large sack chopped straw } + 6 oz. sulphate of ammonia.
One large sack dried lawn mowings }
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
16 days composting. Max. temperature 148° F.

Compost ...	VII +l. −s.	VIII −l. −s.	IX +l. +s.	X −l. +s.	
pH	8·02	8	7·57	7·57	5 g. superphosphate of lime added per pot, and watered in after spawning
Origin of culture	Single spore	Single spore	Stipe	Stipe	
Fructification	−	−	+ +	+	
			Pl. XV, fig. 8		

Division III. Composts XI–XIV.

One large sack chopped hay } + 10 oz. sulphate of ammonia.
One large sack dried lawn mowings }
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
20 days composting. Max. temperature 160° F.

Compost ...	XI +l. −s.	XII −l. −s.	XIII +l. +s.	XIV −l. +s.	
pH	8·6	8·57	8·72	8·92	Watered with ½ l. 0·01% oxalic acid after spawning
Origin of culture	Not tested	Stipe	Stipe	Stipe	
Fructification	−	−	−	+	

+l. with lime; −l. without lime. +s. with soil; −s. without soil.
+ fructification; − no fructification.

exposed to the air (Hein, 1930*c*), and hence it was thought that the mushroom might possibly be able to use free oxalic acid. Onslow states (1931, p. 113) that Dorée & Barton Wright (1927) have suggested a constitutional formula for alkali lignins, and that they maintain, that in

Table II

Artificial composts. Naturally rotted in the open without heat for 3–4 weeks. Pot cultures spawned with cultivated varieties

Division IV. Composts XV–XVIII.

One large sack chopped straw, One large sack chopped hay } +6 oz. sulphate of ammonia.
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
Not turned.

Compost ...	XV	XVI	XVII	XVIII	
	+l. −s.	−l. −s.	+l. +s.	−l. +s.	
*p*H	8·12	7·93	7·25	7·28	5 g. superphosphate of lime added per pot, and watered in after spawning
	8·07	7·9	7·3	7·28	
Origin of culture	Same stipe culture throughout				
Fructification	+	+	+ +	+	

Division V. Composts XIX–XXII.

One large sack chopped straw, One large sack dried lawn mowings } +6 oz. sulphate of ammonia.
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
Not turned.

Compost ...	XIX	XX	XXI	XXII	
	+l. −s.	−l. −s.	+l. +s.	−l. +s.	
*p*H	7·56	7·43	7·5	7·1	No superphosphate added
	7·53	7·36	7·46	7·08	
Origin of culture	Same stipe culture throughout				
Fructification	+	+	+	+	

Division VI. Composts XXIII–XXVI.

One large sack chopped hay, One large sack dried lawn mowings } +6 oz. sulphate of ammonia.
Half without lime, half with 4 oz. slaked lime.
Equal volume of soil added to those composts with soil.
Not turned.

Compost ...	XXIII	XXIV	XXV	XXVI	
	+l. −s.	−l. −s.	+l. +s.	−l. +s.	
*p*H	7·86	7·73	7·32	7·17	No superphosphate added
	7·77	7·68	7·32	7·17	
Origin of culture	Same stipe culture throughout				
Fructification	+	+	+	+	

Symbols the same as in Table I.

such a structure, decomposition by oxidation would probably give rise to general disruption and the production of oxalic acid and carbon dioxide.

In spite of the extreme stickiness of the compost, the "Honeymoon" variety produced small pilei after prolonged growth in Compost XIV (Table II) to which oxalic acid had been added, but doubtless the free oxalic could not have remained as such for any length of time.

No growth occurred in Composts XI, XII, and XIII.

On the other hand, the same mixture (chopped hay and dried lawn mowings) naturally rotted without heat, showed lower alkalinity, was not treated with oxalic acid or superphosphate and the cultures produced normal pilei (Pl. XVI, fig. 27).

The addition of garden soil was found to reduce the alkalinity of both fermented and naturally rotted composts, with the exception of the fermented composts in Division III (Table I). Here the *p*H is raised by the addition of soil.

p*H* OF COMPOSTS BEFORE AND AFTER STERILIZATION

These Division III composts were tested before and after sterilization, and it was found that the *p*H was raised by sterilization in composts without soil (Table I, Composts XI and XII), and more markedly in Compost XIV with soil but without lime.

In Division II (Table I) Composts IX and X were also tested before and after sterilization, and here again the *p*H was slightly reduced in both cases (Table III).

Table III

pH of composts before and after sterilization

Composts	Unsterilized	Sterilized
IX. Chopped straw, dried lawn mowings +l. +s.	7·57	7·4
X. Chopped straw, dried lawn mowings −l. +s.	7·57	7·53
XI. Chopped hay, dried lawn mowings +l. −s.	8·6	8·86
XII. Chopped hay, dried lawn mowings −l. −s.	8·57	8·74
XIII. Chopped hay, dried lawn mowings +l. +s.	8·72	8·7
XIV. Chopped hay, dried lawn mowings −l. +s.	8·71	8·92

AGE OF SPAWN

It will be seen in the explanation of the plates, p. 339, that spawn of varying ages was used in these compost tests, ranging from 2 months to 9 months and 3 weeks. In the pot and box cultures all the spawn was grown in the laboratory under sterile conditions on the standard spawn compost with the exception of the box in Pl. XV, fig. 9. In the pots of fermented compost in Pl. XV, figs. 1, 2, the spawn was 8 months 10 days old, fig. 3, 2 months 1 day old, and fig. 10, 7 months 11 days old, showing that, when grown in this medium, spawn remains viable for a considerable period and that the age (within these limits) has not affected the resulting crop.

MYCELIAL DEVELOPMENT IN DIFFERENT COMPOSTS

Different physical conditions in these artificial composts greatly affect the type of mycelial growth. The standard spawn compost, consisting of chopped straw, chopped hay, crushed oats and Styer's nutrient solution "A" (1928), sterilized fresh and not submitted to any decomposition by micro-organisms, is a loose open compost in which the mycelium is fine and dense, spreads evenly and rapidly, and thoroughly permeates the compost. If not allowed to dry out, the spawn will remain in the diffuse filamentous condition for a considerable period without strand formation. Again, in high- and low-temperature composts without soil, strand formation is delayed. Pl. XVI, fig. 14 is a culture of commercial spawn in a 2 months low-temperature compost after 10 weeks growth, and fig. 16 a culture of the "Honeymoon" cultivated variety in the same compost after 18 weeks growth. In the latter culture, with the exception of the course fructifying strand, the mycelium is still diffuse.

The addition of soil to high-temperature and naturally rotted composts sets up entirely different physical conditions. The composts are denser and of a more homogeneous nature, with the result that the mycelium tends to develop strands in a comparatively short time, and, hence, these composts are useless for spawn growing. Pl. XVI, figs. 11 and 12, are cultures of the cultivated mushroom in a high-temperature compost with and without lime but both containing soil in which strand formation has occurred after 11 weeks.

The effect produced by the addition of soil to naturally rotted composts is still more marked in the case of the wild *Ps. campestris*, a soil-inhabiting species. Pl. XVI, figs. 17–20, is a series of cultures of *Ps. campestris* after 3 months growth, figs. 17 and 18 without soil, figs. 19 and 20 with soil. Strand formation has occurred in the cultures with soil and not in those without.

The addition of lime together with soil tends to produce strand formation both with the cultivated varieties and with *Ps. campestris* (cf. Pl. XVI, figs. 12, 13, and 19, 20).

The absence of lime in both fermented and naturally rotted composts induces a tendency to clumping of the sporophores of the cultivated mushroom (Pl. XV, figs. 2, 7: Pl. XVI, fig. 25).

Owing to lack of adequate cultural conditions no reliable data are available as to the length of the period of incubation with the various composts, but in general, pilei of the cultivated varieties developed more

rapidly and were of better consistency on fermented than on naturally rotted composts. As regards texture, the pilei produced on artificial composts are, as a rule, neither so firm nor so heavy as on manure, although occasionally firm pilei have been obtained on fermented composts in medium bulk in the boxes.

The commercial varieties vary in flavour when grown on manure. The same is the case on artificial composts. The "Honeymoon", the best flavoured variety used in these experiments, retains its delicate flavour when grown on artificial composts.

Discussion

The investigation of artificial composts is still in the experimental stage and no claim is made that the results are, as yet, of any commercial value, except in so far as spawn growing is concerned. The standard spawn compost is cheap, easy to make and has the advantage of delaying the transition from diffuse filamentous mycelial growth to strand formation and thus enables the spawn to be kept in a suitable condition for a prolonged period. Spawn in which stringy growth predominates is known to give poor results.

The problem of the nutritional requirements of the mushroom and its reactions to the physical conditions in the substratum is so complex that it is impossible to attribute any phenomenon to any one cause or factor; but these results show definitely that the various composts, both fermented and naturally rotted, with and without lime, contain the necessary nutrients for the growth of the cultivated mushroom, and that, in spite of very inadequate cultural conditions, normal pilei developed in the majority of the mixtures tested.

The main difficulty throughout has been to secure favourable physical conditions, i.e. adequate aeration and friability, and possibly, as described by Pizer (1937*b*) for stable manure, some physicochemical condition. Samples of artificial composts of sticky consistency, which had given poor results, were submitted to Pizer, who pronounced the opinion that stickiness in artificial composts was quite a different property to "greasiness" in stable manure, and that the samples submitted to him had not been sufficiently composted. The latter is probably true, but these composts were not made primarily with the object of devising a suitable compost for growing the commercial cultivated varieties, but in order to confirm previous tests as to the reactions of the two wild grassland species *Ps. campestris* and *Ps. arvensis* and the wild haystack mushroom to fermentation of short duration; the cultivated varieties were only

used as controls to test the physical condition and the nutritional value of the various mixtures.

The stickiness in fermented artificial composts is most marked in the richer mixtures containing dried lawn mowings, both with and without soil, but is considerably less in mixtures of chopped straw and hay. The addition of an equal volume of soil to the latter mixture resulted in a good friable compost, but this was only tested in pots and boxes and not in greater bulk.

Unfortunately, Pizer's method for improving greasy stable manure by adding gypsum at certain stages during the process of composting had not been published when these composts were prepared, and it remains to be seen whether this method would alter the physical condition of sticky artificial composts. If adequate physical and physico-chemical conditions can be induced it should be possible, after further experiment, to produce a compost containing the same ingredients capable of carrying a crop equal to that on stable manure. These artificial composts do not retain the heat so long as stable manure and therefore artificial heating of the mushroom houses would be necessary if quick results are to be obtained. For instance, Pl. XV, fig. 9, shows the second flush on a compost fermented at a high temperature for 17 days and kept during the incubation period between spawning and cropping in an intermediate greenhouse. Under these warmer conditions the compost retained the heat for a much longer period. The first flush appeared 10 weeks after spawning and the second about 2 weeks later. On the other hand, in the culture in Pl. XV, fig. 10, of home-grown spawn of a white market variety kept in a cold cellar throughout, the first flush only appeared after 5 months 11 days.

The effect on mycelial growth produced by the addition of an equal volume of soil to the composts requires further investigation. The first effect is to reduce the temperature, the second to induce early formation of strands, and it is quite possible that by reducing the amount of soil a mixture could be produced in which strand formation would be sufficiently delayed to allow of the compost being thoroughly permeated with vigorous filamentous mycelium before strand formation and fructification.

Hein (1930*c*), Pizer (1937*b*) and Styer (1930) have observed that strand formation predominates in composts with too high moisture content. The early strand formation in the battery jars figured in Pl. XVI cannot be attributed to excessive moisture, as the cultures were kept on the dry side during the early stages of growth, but rather to the

reduced aeration in the denser composts containing soil; or possibly to some unknown ingredient in the soil which stimulates a more rapid rate of metabolism at the expense of diffuse vegetative mycelial growth.

When plated on nutrient agar the submerged mycelium produces few or no crystals, but the aerial mycelium is thickly encrusted with crystals of varying length and arrangement, mainly calcium oxalate (Hein, 1930*b*), and the drier the conditions the denser the mycelium and the whiter the appearance. Hein has suggested that the crystals may be a waste product of mycelial metabolism.

Crystals of calcium oxalate are only slightly soluble in water, and a possible explanation of the deleterious effects of excessive moisture may be, that under inadequate aeration together with too much moisture, the mycelium cannot get rid of waste products by crystallization. Hence the spread and rate of growth is restricted and the mycelium passes over into the next developmental phase, i.e. strand formation, before having derived the full benefit of the nutrients in the compost or accumulated and stored sufficient food material for normal fructification, with the result that the pilei are undersized or do not reach maturity.

The aeration of a compost in a glass container must necessarily be poor and not conducive to the development of normal life cycle, more especially after prolonged growth. Hence, although in a number of cultures the compost became thoroughly permeated with mycelium, only occasional pilei of the cultivated varieties have been obtained in battery jars.

The cultivated varieties differ somewhat from the wild grassland species with respect to moisture conditions during mycelial development. In well-composted, well-aerated stable manure, moist heat is conducive to rapid mycelial growth, and in the battery jars, under cooler conditions, it was found that the cultivated varieties would tolerate—within limits—a higher moisture content than the two wild grassland species.

As regards temperature, on the other hand, it is a well-known fact that in districts where the wild field mushroom is prevalent, hot dry weather in July and August followed by rain in September results in a heavy crop, whereas after a cool wet summer few or no mushrooms can be found. The wild field mushroom is mostly found in well established turf on heavy soils retentive of moisture and considerable drying out may be necessary for adequate aeration.

These observations suggest that of the two main interacting factors, moisture content and aeration, both of which affect the physical condition of the substratum, the presence of too much moisture is instru-

mental in reducing or inhibiting crystallization, and aerobic conditions are necessary for metabolism and growth.

The marked reduction of the lignin content in composted stable manure observed by Waksman and his co-workers, and the question as to how it is utilized by the mushroom are still unexplained. With naturally rotted straw and hay composts which have not undergone high temperature fermentation, the above described experiments show that previous high-temperature fermentation is not absolutely essential for normal growth, although the gradual disintegration by micro-organisms and by the mushroom itself during the incubation period may help to increase the relative lignin content to some extent. The two media—stable manure, some ingredients of which have been subjected to animal digestive juices, and naturally rotted straw and hay must necessarily be entirely different both physically and chemically and perhaps are hardly comparable, but the examination of the plant residues in the artificial composts during mycelial growth has shown that the fungus penetrates the tissues, the mycelium is mainly *intra*-cellular and that the parenchymatous cells are the first to be attacked. The lignified tissues are only penetrated after prolonged growth. Lignin is present in the xylem parenchyma, but not in sufficient quantity for a lignin destroying fungus requiring a considerable amount of lignin as such. The expectation would be, *inter*-cellular mycelium, a heavily infected vascular system and rapid disintegration of the plant residues in the compost. This does not occur in sterilized artificial composts.

Artificial infections of sterile grass seedlings have shown that all the forms of mushroom under investigation can penetrate the living roots. The parenchymatous cells of the cortex are attacked and become completely filled with coiled mycelium, but the vessels remain more or less free.

Again, experiments described in a previous paper (Cayley, 1937) show definitely that the two wild grassland species will not grow on either fermented manure or any fermented artificial compost so far tested, but spawn grows quite freely on unfermented freshly sterilized straw and hay.

These results indicate that mainly proteins, hemicelluloses and cellulose are required by the fungus during the earlier stages of growth, but that lignin or lignin derivatives may possibly be necessary for fructification, although Waksman & McGrath (1931) found very little lignin in the pileus itself (0·92 % dry material) as compared with hemicellulose (13·66 %) and cellulose (4·86 %).

Summary

Methods for composting fermented and naturally rotted artificial composts consisting of straw, hay and dried lawn mowings for the growth of mushrooms are described.

The results of the tests in large pots and boxes on twenty-four different composts are set out and show that previous high temperature fermentation is not absolutely essential for the growth of the cultivated mushroom, although the best results have been obtained from high-temperature composts containing both lime and soil.

The effect of the addition of soil to both fermented and naturally rotted composts is described and discussed.

In conclusion I wish to express my indebtedness to Dr A. C. Fabergé for all the pH estimations, to Miss F. M. Durham for testing high-temperature Compost I in the open, and to the laboratory assistant, A. F. Emarton, for the photographs for the plates and for his invaluable help with the preparation and manipulation of the various composts and cultures without which such a number of tests would not have been possible.

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EXPLANATION OF PLATES XV AND XVI

PLATE XV

FERMENTED COMPOSTS

High-temperature composting for 17 *days*

Compost: chopped straw, chopped hay, sulphate of ammonia, lime and superphosphate. 4 months after spawning.

Fig. 1. White market variety (1), first flush, Compost III. Spawn 8 months 10 days old.
Fig. 2. White market variety (1), first flush, Compost IV. Spawn 8 months 10 days old.
Fig. 3. "Honeymoon" variety, first flush, Compost V. Spawn 2 months 1 day old.
Fig. 4. "Honeymoon" variety, second flush, Compost V. Spawn 2 months 1 day old.
Fig. 5. "Honeymoon" variety, third flush, Compost V. Spawn 2 months 1 day old.
Fig. 6. "Honeymoon" variety, first flush, Compost VI. Spawn 2 months 1 day old.
Fig. 7. "Honeymoon" variety, second flush, Compost VI. Spawn 2 months 1 day old.

High-temperature composting for 16 *days*

Compost: chopped straw, dried lawn mowings, sulphate of ammonia and superphosphate. 5 months 10 days after spawning.

Fig. 8. White market variety (2), first flush, Compost IX. Spawn 9 months 21 days old.

High-temperature composting for 31 *days*

Compost: long straw, long hay, chopped straw, chopped hay, lime, sulphate of ammonia and phosphate of potash. 3 months after spawning.

Fig. 9. Bought white commercial spawn, second flush, age of spawn unknown. Compost I.

High-temperature composting for 17 *days*

Compost: chopped straw, chopped hay, sulphate of ammonia and superphosphate. 5 months 11 days after spawning.

Fig. 10. White market variety (3), first flush, Compost III. Spawn 7 months 11 days old.

PLATE XVI

STERILIZED COMPOSTS IN BATTERY JARS

High-temperature composting for 17 *days*

Compost: chopped straw, chopped hay, and sulphate of ammonia.

Fig. 11. White market variety (2), Compost V, after 7 months growth.
Fig. 12. White market variety (2), Compost VI, after 7 months growth.

Natural rotting for 28 *days, without heat*

Compost: chopped straw, chopped hay, and sulphate of ammonia.

Fig. 13. White market variety (3), Compost XVII, after 2 months 3 weeks growth.

Low-temperature composting for 2 months

Compost: chopped straw, chopped hay, and sulphate of ammonia.

Fig. 14. Commercial spawn, after 2 months 2 weeks growth.
Fig. 16. "Honeymoon" variety, after 4 months 2 weeks growth.

Composted stable manure, without lime

Fig. 15. Commercial spawn, after 2 months 2 weeks growth.

Natural rotting for 3 weeks, without heat

Compost: chopped straw, chopped hay, and sulphate of ammonia.

Fig. 17. Wild field mushroom, *Ps. campestris*, Compost XV, after 3 months growth.
Fig. 18. Wild field mushroom, *Ps. campestris*, Compost XVI, after 3 months growth.
Fig. 19. Wild field mushroom, *Ps. campestris*, Compost XVII, after 3 months growth.
Fig. 20. Wild field mushroom, *Ps. campestris*, Compost XVIII, after 3 months growth.

Unfermented, unrotted standard spawn compost

Compost: chopped straw, chopped hay, crushed oats and nutrient solution, without lime or soil.

Fig. 21. Wild field mushroom, *Ps. campestris*, after 2 months growth.
Fig. 22. Wild horse mushroom, *Ps. arvensis*, after 2 months growth.

POT CULTURES

Natural rotting for 3 weeks, without heat

Compost: chopped straw, chopped hay, sulphate of ammonia and superphosphate. 6 months onwards after spawning.

Fig. 23. "Honeymoon" variety, first flush, Compost XVII. Spawn 5 months 17 days old.
Fig. 24. "Honeymoon" variety, second flush, Compost XVII. Spawn 5 months 17 days old.
Fig. 25. "Honeymoon" variety, first flush, Compost XVIII. Spawn 5 months 17 days old.

Compost: chopped straw, dried lawn mowings, sulphate of ammonia but *no* superphosphate.

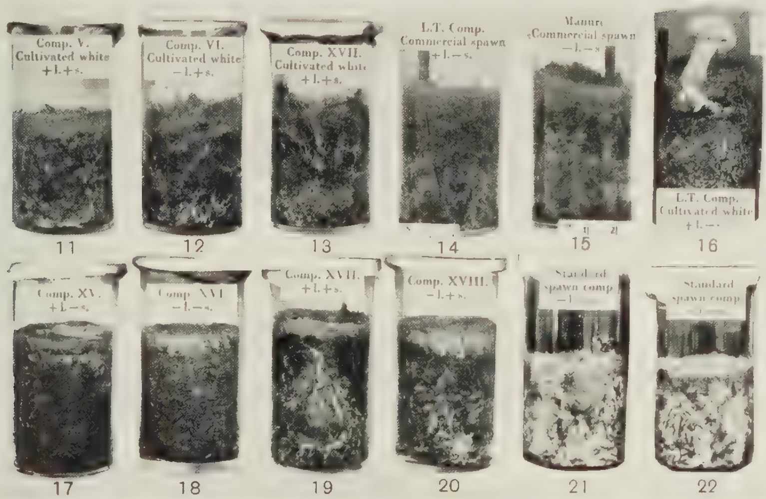
Fig. 26. "Honeymoon" variety, first flush, Compost XX. Spawn 5 months 16 days old.
Fig. 28. "Honeymoon" variety, first flush, Compost XXII. Spawn 5 months 16 days old.

Compost: chopped hay, dried lawn mowings, sulphate of ammonia but *no* superphosphate.

Fig. 27. "Honeymoon" variety, first flush, Compost XXI. Spawn 5 months 16 days old.

(*Received* 2 *November* 1937)

Fermented high temperature composts.



Spawn on various media.

Composts naturally rotted without heat.

CAYLEY.—Experimental Spawn and Mushroom Culture (pp. 322–340)

FIELD EXPERIMENTS ON THE CONTROL OF WIREWORMS

By W. R. S. LADELL

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With Appendix

THE INFORMATION SUPPLIED BY THE SAMPLING RESULTS

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Statistical Department, Rothamsted Experimental Station

(With 7 Text-figures)

CONTENTS

I. Introduction

King & Glen (1933), reporting progress on wireworm experiments in Saskatchewan, stated "while the necessity for replication is recognized, there seems to exist no basis upon which to determine what might constitute adequacy of replication in respect to the known variability of

wireworm infestation". Further, "preliminary statistical analysis indicates that the methods used for making an annual census of wireworms are reasonably adequate, but final conclusions on this point must await much further study and appraisal". More recently, Jones (1937) compared the figures obtained from sampling units of three sizes and has calculated the errors in sampling at different population densities. The purpose of the present paper is to present the results of three soil fumigation experiments carried out against wireworms on a field scale and using a special field plot technique. It is our object to demonstrate the adequacy of replication required for such types of investigation rather than the value of any particular fumigant.

There are many difficulties in carrying out field trials involving the use of soil fumigants against wireworms. In the first place, wireworms, although easily recognized as such, cannot be readily separated into their correct species. Further, the habits of wireworms as regards the depth at which they are found at different seasons of the year and under different weather conditions are only vaguely understood. This uncertainty is reflected in the different depth and size of the soil samples used by various investigators, such as Roebuck (1924), Miles & Petherbridge (1927), Miles (1932), King (1928, 1929), Lane (1933), Hawkins (1934, 1936 *a*, *b*), Lane & Jones (1936), and Lacroix (1935). Other major difficulties are the choice of a site and the separation of the wireworms from the soil. In connexion with the former, it is sometimes a troublesome task to find exactly when wanted an area which is large enough for the experiment and sufficiently infested with wireworms. The method of separation of the wireworms from the soil must be sufficiently accurate to obtain even the smallest wireworms which, owing to the long life cycle, are found simultaneously with older ones, and sufficiently rapid to allow the samples to be examined before desiccation has caused any diminution in numbers. The effect of the fumigants on the crop must also be examined critically.

Before dealing with the experiments, it is proposed to consider the distribution of wireworms in the field, and then to describe in some detail the methods used in estimating the wireworm population found in the experiments.

While the literature on the prevalence of wireworms, their damage and methods for their control is voluminous, that on sampling technique and properly replicated field experiments designed to show the value of fumigants or other treatments against wireworms is scanty. Reference should be made to Jones (1937), who has recently reviewed the literature

from this point of view, and to Thomas (1930), who reviewed the published research on the control of wireworms.

II. Distribution of wireworms in the field

It is commonplace knowledge that wireworms are not uniformly distributed throughout a field, yet care has not often been adequately taken to ascertain their distribution. The reasons for this apparently random distribution are not understood, but no doubt it depends to a large extent on the preference of the wireworms for certain soil conditions and the availability of the requisite food. Thus, it is frequently stated that wireworms in their early instars feed chiefly on humus and only later attack living roots. In the first experiment, the pH of all the soil samples was taken with a view to finding out if there was any relation between this factor and the distribution of the wireworms. The pH varied considerably, from 5·8 to 6·8 with a mean of 6·3, but no

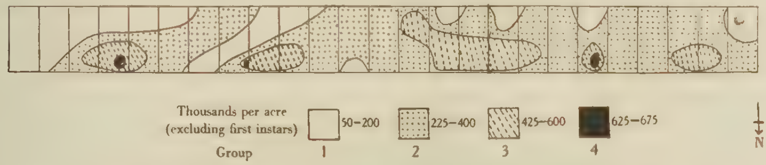


Fig. 1. Preliminary survey of wireworm population in area of old grassland before ploughing up for tests of soil fumigants.

relationship between the reaction of the soil and the number of wireworms was apparent. Although it is outside the scope of the present paper, it may be pointed out that an exact knowledge of the factors underlying the distribution of wireworms in a field might lead to effective preventive measures being taken against an attack. This would be of great value except where old grassland had to be broken up for cultivation. Under such circumstances the use of palliative methods involving soil fumigants must be of major importance.

Fig. 1 illustrates the distribution of wireworms in a strip of land which had not been cultivated for many years and was covered with moss, weeds and grass. This area was chosen for the Knott Wood experiment to be described later. Three random samples, each 9 in. square with a depth of about 5 in., were taken from each half-plot, making a total of 150 samples in all, and the numbers of the wireworms are given in Table I. It will be seen that the distribution is anything but uniform. Emphasis must be laid on the fact that this indicates the distribution of the wireworm population on the date of sampling, March 1935, only.

Table I

Knott Wood experiment. Preliminary survey: before fumigation

No. of wireworms in soil samples $9 \times 9 \times 5$ in.

Serial no. of plot	South half	North half	Totals S	Totals N	S+N	(S−N)
103	0 2 0	2 2 0	2	4	6	2
104	1 0 1	1 2 1	2	4	6	2
105	1 1 0	4 4 10	2	18	20	16
106	2 4 1	6 7 14	7	27	34	20
107	0 5 1	2 3 8	6	13	19	7
108	0 0 4	4 3 4	4	11	15	7
109	5 2 5	3 0 5	12	8	20	4
110	1 5 4	4 9 11	10	24	34	14
111	1 1 5	9 8 1	7	18	25	11
112	2 4 2	5 4 9	8	18	26	10
113	2 8 0	4 5 1	10	10	20	0
114	4 2 3	6 1 1	9	8	17	1
115	4 8 3	5 2 2	15	9	24	6
116	4 5 11	4 4 5	20	13	33	7
117	0 2 2	7 8 3	4	18	22	14
118	6 5 5	8 7 8	16	23	39	7
119	2 0 3	9 3 7	5	19	24	14
120	9 1 3	4 9 10	13	23	36	10
121	5 2 5	4 4 4	12	12	24	0
122	0 4 3	10 11 4	7	25	32	18
123	3 4 3	12 2 2	10	16	26	6
124	3 4 5	0 3 7	12	10	22	2
125	1 5 9	6 6 8	15	20	35	5
126	5 4 2	5 7 6	11	18	29	7
127	4 0 4	5 4 2	8	11	19	3

Standard errors per plot.
Sampling error $\pm 6{\cdot}06$ or 25%.
Experimental error $\pm 6{\cdot}99$ or 29%.

III. Technique used in assessing the wireworm population by direct counts on soil samples

Soil sampling

It was obvious that extensive soil sampling was essential in order to obtain accurate figures of wireworm infestation. The *size* of the sample used was at first 9 in. square to a depth of about 5 in. Such a sample weighs 20–27 lb. Later, consequent on an improvement in the technique of extracting the wireworms from the samples, a smaller sized sample was used. These later samples were 6 in. cubed and weighed 9–12 lb. The *number* of samples taken in the first experiment was six per plot. Later this number was reduced to four, and in the last experiment (when the size of the plot was only $\frac{1}{200}$ acre) to only two samples per plot.

Where possible, local control was instituted by taking an equal

number of samples in each half-plot. The *time* for sampling was immediately before fumigation and again a few weeks afterwards.

The *position* or *location* of a sample was found by measuring two coordinates from the corner of each plot and was determined beforehand, without any choice on the part of the sampler, by reference to a book of random numbers. This absence of selection on the part of the sampler is essential to obtain a proper estimate of the wireworm population in the area, though this has often been overlooked in previous counts of wireworms. Numbered wooden pegs were used to indicate the positions of the samples in the field.

The *depth* of sample was determined by preliminary sampling which showed that in grasslands the great majority of the wireworms were to be found in the top 4 in. of soil. In order to reduce the amount of soil to be examined, it was decided to use that depth as a standard, and although the figures so obtained would be slightly lower than the truth they would be comparable amongst themselves. In practice it was found that it was impossible to restrict the depth to an accurate 4 in. as the turn-over of the soil sampling tool then used would penetrate to a greater or less depth according to whether the soil was wet and soft, or dry and hard. If the tool was hammered right into the soil so that the top of the sampler was on a level with the ground the depth was 5 in., and it was easier to maintain this as a standard sample. Later it was found that although 5 in. was sufficiently deep for the preliminary sampling on grassland it was not so satisfactory for the second sampling, for which a depth of 6 in. was better, as the soil sample would then include all the partially decomposed grass of the furrow slice and surviving wireworms feeding on the grass roots. Therefore the latest tool was designed to give a sample to a depth of 6 in. when the top was hammered level with the surface of the ground.

Type of soil. The Rothamsted soil is derived from clay-with-flints material and lies over chalk. The irregular presence of flints and the high clay content, up to 30 %, makes the task of soil sampling and the subsequent examination a matter of great difficulty, and for these reasons a special tool had to be designed for sampling.

Sampling tool. This is made of $\frac{1}{4}$ in. mild steel plate sharpened and bent to form two sides at right angles with the back, each side being 6 in. long. The top of the plate is turned over externally to give an overlap of 1 in. and the distance from the bottom of this to the cutting edge is 5 in. making a total of 6 in. The tool is hammered into the ground with a "beetle" made of applewood or witch-elm bound with hoop iron. A

straight piece of steel plate is then hammered into the soil across the front of the tool in order to cut the sample. The latter is lifted by slipping a spade underneath from the back while a second operator raises the tool by means of hooks placed in holes at the sides. One operator can, however, take soil samples by the expenditure of a little more time. If possible the soil is kept unbroken to avoid too rapid drying out. On grass or pasture land the grass or weeds are cut close to the ground before the sample is taken.

The samples thus obtained are put into separate canvas or jute sacks together with the numbered peg previously used to indicate the position of the sample in the field and a paper label. Another paper label was fixed externally as an extra precaution, and to assist in arranging the samples for examination.

Preliminary preparation of samples

This process took anything from 15 to 45 min. depending upon the state of the soil. The soil sample was first broken up by tearing it upon a hackle. This is a block of wood with oval nails projecting $1\frac{1}{2}$ in. through it. At one end of this block the nails are set 2 in. apart and at the other end 1 in. The block is fixed to the bench with large "G" clamps. A tray under the bench receives the soil as it falls from the hackle. The wider spaced nails are first used, and then the resulting small sods of soil are broken up still further on the 1 in. spaced nails. Next the grass and weeds are separated as far as possible from the soil which is rubbed through a garden riddle of 3 meshes to an inch. Each portion of soil was examined for wireworms as it came through the riddle. In some of the earlier counts the soil was placed on sheets of brown paper, spread out and searched for any remaining wireworms. The grass and weeds were kept separate and made into parcels for later treatment.

Examination of samples

As has been previously stated, the soil from the samples was at first placed on sheets of brown paper for the final examination. This was found unsatisfactory and recourse was made to a flotation process used in an apparatus devised by the writer (1936) for the separation of insects from the soil. This apparatus could not be used for the experiments described in this paper owing to the large size of the samples.[1] Instead,

[1] A larger machine has been put on the market by Messrs A. Gallenkamp and Co., which will take a 6 in. cube of soil. This machine was used for the most recent sets of samples, which, after a preliminary hackling and riddling, were dealt with quickly and efficiently (see p. 348).

the soil was tipped into a 5 gal. oil drum filled to within 3–4 in. of the top with a solution of magnesium sulphate (density 1·11: about 25 % of the commercial salts). Stirring was done by means of wooden stakes for 3 min. After an interval of at least 5 min. the debris was skimmed off the surface by means of a fine mesh strainer (40 meshes to the inch), any adhering to the sides of the drum being removed by the use of a brush. All this debris was washed twice with warm water, squeezed out thoroughly by hand and placed in a tin for further examination later. The stirring and skimming was repeated four or five times until no more material rose to the surface.

The flotation solution may be used over and over again after decanting from the mud and being made up to the necessary strength. The parcels of grass and weeds from the preliminary preparation of the samples were soaked in warm water in a basin for 5 min. and squeezed out, thus freeing them from any adhering soil. This process was repeated and the resulting clean grass and weeds were put into a further tin for later examination. The soil from the grass and weeds was dealt with similarly to the original soil, that is to say, washed through a strainer and any residue was placed in the first tin. Usually the paper label from inside the sample sack was left to mark the oil drum, the wooden label placed in the second tin, i.e. that containing the grass and weeds, while the tie-on paper label from the sack was placed in the first tin, i.e. that containing the debris which was floated.

Examination of floated debris. Naturally most of the wireworms were found in the debris which had been floated up in the solution and this required critical examination. This was done by teasing the debris apart with needles and examining it under a low-power lens mounted on a tripod. Most of the wireworms being alive and active[1] were easily seen; but in some cases where the soil sample had dried out before being dealt with a few dead wireworms occurred and these were not so easily detected.

[1] In the U.S.A., magnesium sulphate is reported by Frings & Frings (1937) to have insecticidal properties, and by Hawkins (1936 *b*) actually to be toxic to the wheat wireworm, whereas I have always found it non-toxic in my laboratory experiments. After immersion all night in the strong solution of magnesium sulphate (25 % of the commercial salts) used in my machine, wireworms were still alive. They were often stiff and rigid but soon recovered after being taken out of the solution. This discrepancy in observations might have been due to a higher temperature in the U.S.A. experiments, to some other important difference in procedure, or merely due to the different species of insect. However, since then, R. C. Smith (1937), of the Kansas Agricultural Experimental Station, has proved that the claims of Frings are unjustified, and that "the small test by Hawkins against the wheat wireworms mentioned by the Frings was apparently not regarded as significant by him, for he drew no practical conclusions from it".

In order to obtain some idea of the different instars being encountered, the wireworms were measured. A cover-slip was used to straighten them before measurement. Six arbitrary groups of $<0{\cdot}5$, 0·6–0·9, 1·0–1·3, 1·4–1·7, 1·8–2·0, and >2 cm. were employed.

Use of Ladell machine (1936) *for separating wireworms from the soil*

Preliminary examination. With a sandy soil such as the Woburn type, the soil sample can be put straight into the machine, but with the clayey soil characteristic of Rothamsted a special technique is required. A further modification is necessary to cope with the mass of undecomposed grass that was present in the soil samples taken from High Field after fumigation.

The soil sample weighing 12–14 lb. is broken up by means of a small garden fork or by the use of the hackle mentioned above. If not too sticky the soil is then passed through a garden riddle. Stones are removed as far as possible. The grass with any adherent soil is soaked in a small quantity of the magnesium sulphate solution (sp. gr. 1·11), sufficient to cover it. The grass is torn apart and rubbed by hand underneath the solution until all the mud is detached, then squeezed and washed in a similar fashion with a further quantity of magnesium sulphate solution. The two lots of muddy solution are mixed together and poured into the machine with the prepared soil.

The grass is washed under running water over a sieve and may be discarded, but the debris on the sieve should be examined for wireworms.

Main process. The main process is as follows: The muddy liquid from the grass is poured into the cylinder with sufficient clean solution to bring the level of the liquid up to the bottom sieve. The soil is then tipped on to the sieve and more solution added until the top sieve is reached. The head is then clamped on, and the cylinder placed in position on the stand, the inlet pipe is connected with the reservoir and more solution allowed to run in. The air pump is connected and switched on, and the stirrer started. After 10 min. the machine is stopped and the liquid allowed to stand for 5 min. All the froth is floated off and then the tap on the cylinder is opened to allow about one-third of its contents to flow away. Afterwards the level of the liquid is brought back to the top of the conical head.

The stirring and bubbling is then continued for a further 2–5 min., the froth being floated off across the surface of the liquid in the tank and collected on a brass sieve with 40 meshes to the inch. The debris on

the sieve is washed twice with water, squeezed out thoroughly by hand and placed in a tin.

This debris is teased apart on a white tray with entomological needles and examined under a low-power lens supported on a tripod. The wireworms are divided into six groups according to their length, as previously stated.

Discussion of the sampling methods used

In an extensive sampling of field plots for wireworms there is great difficulty in examining the large number of soil samples quickly, and this is particularly evident when dealing with the intractable clayey soil of Rothamsted. The first sampling of any experiment should be done immediately before fumigation, but if all the samples are taken on the same day the number is too great to be completed within a reasonable period, and there is danger of serious deterioration of the samples on keeping. As an alternative, the sampling may be spread over a week or two before fumigating, as long as complete blocks are sampled on the same day. This might affect variation between blocks, but not variation within blocks from which the experimental error is derived.

Another difficulty inherent in this type of soil is due to its water-holding capacity. Soil samples taken in the spring are saturated with water and cannot be examined without preliminary drying. For this purpose the sacks containing the sods are hung up on rails with the grass side uppermost, the air circulating round the sacks being sufficient to keep the soil from going sour before it can be examined. Should a reductive phase set in the earlier instars of the wireworms are liable to die. In the cool weather of the spring, the wireworms will remain quiescent in the grass sods for several weeks and none will be lost, but as the weather gets warmer, and especially if the soil dries out too quickly, the wireworms and predators become active and the result is loss by cannibalism or destruction by staphylinid or carabid larvae. These difficulties were all minimized when the size of the soil sample was reduced from 9 to 6 in. square, and the hand examination was superseded by the flotation technique which made it possible to work with the soil while it was still wet.

The second sampling undertaken a few weeks after fumigation was nearly always done in blocks, and the examination of one block completed before the next block was sampled, the reason being that the wireworms were always more active at this later date, the soil drier and not carrying any living grass. Thus the samples had to be examined as

quickly as possible to avoid loss of wireworms. Table IXA is included to show that this extended sampling is not prejudicial to the results. There is no progressive falling off of numbers from the earliest to the latest dates of sampling.

Only in one case was there any serious loss of numbers, and that was with the first sampling of High Field I. The sampling was done very early in the year, at the end of January, and the soil was exceedingly wet. The samples from blocks VII–X, Table VII, had been taken several weeks when a sudden spell of warm weather threatened to dry out the soil too rapidly. One sample from each plot was examined at once and the others moistened. On examining the other three samples from these plots the numbers were found to be almost consistently below those of the first samples, and there was evidence of cannibalism and destruction of wireworms by predators. It was a question of basing the results on one sample only in these plots, or applying a correction to the actual figures obtained. The latter course was the more accurate. The correction was calculated by W. G. Cochran, using the method of least squares, and was applied to the figures from the three moistened samples. Taking as an example block VII "S" and calling the four samples (*a*), (*b*), (*c*), (*d*), (*a*) is the standard sample while (*b*), (*c*) and (*d*) are those that had

Table II

Sampling and experimental errors

Date of sampling	Wireworms per sq. yd.	Size of sample in.	No. of samples	Fraction of plot sampled %	% error per plot: Sampling	% error per plot: Experimental
Knott Wood. 5 × 5 Latin square. Plots $\frac{1}{60}$ acre						
March 1935 Before fumigation	65	9 × 9	3 per ½ plot	0·46	25	29
June 1935 After fumigation	38	9 × 9	2 per ⅓ plot	0·46	34	50
June 1936 Residual effect	45*	6 × 6	2 per ½ plot	0·14	48	60
High Field I. 3 × 10 randomized blocks. Plots $\frac{1}{70}$ acre						
January 1936 Before fumigation	335	9 × 9	2 per ½ plot	0·36	19	21
April 1936 After fumigation	225	6 × 6	2 per ½ plot	0·16	24	34
High Field II. 6 × 8 randomized blocks. Plots $\frac{1}{250}$ acre						
April 1937 Before fumigation	277	6 × 6	2 per plot	0·23	37	36
June 1937 After fumigation	226	6 × 6	2 per plot	0·23	36	31

* Apparent increase due to improvement in technique for separating the wireworms from the soil.

suffered loss. The correction used was $(a)+1{\cdot}763b$ for the first pair of samples and $1{\cdot}763\ (c+d)$ for the second pair:

Actual figures					Corrected figures			
(*a*)	(*b*)	(*c*)	(*d*)	Total	(*a*)	b'	$c'+d'$	Total
28	11	8	8	55	28	19	28	75

A general summary of the sampling details of the three experiments before and after fumigation are given in Table II, including the population density, amount of sampling done, proportion of total area sampled, with the sampling and experimental errors. This table is referred to by Cochran in his discussion of the experiments from the statistical point of view (see Appendix).

IV. Knott Wood experiment

(1) *Lay-out and design of experiment*

For the first fumigation experiment, a strip of land was chosen on the experimental farm between "Pastures" and "Knott Wood" in 1935. This area had not been cultivated for many years and was covered with moss, weeds and grass. Preliminary examination had shown it to be infested with wireworms, mainly *Agriotes sputator* and *A. obscurus*. The land was ploughed and cultivated for sowing with sugar-beet.

The plots were $\frac{1}{60}$ acre in area (40×18 ft.) and arranged as a Latin square but with blocks end to end. (For plan see Fig. 2.) There were four treatments and a control, replicated five times:

	Rate per acre cwt.
Chlordinitrobenzene (*N*)	2·0
Chlorpicrin* (*P*)	2·0
Insecticide *K* (*o*- and *p*-dichlorbenzene) (*K*)	5·0
Insecticide *M* (sodium cyanide and anhydrous magnesium sulphate) (*M*)	7·5

* Adsorbed on Kieselguhr.

4 cwt. superphosphate, 2 cwt. muriate of potash and 1 cwt. ammonium sulphate per acre were applied as basal manuring to all plots. The fumigants, diluted with sand, were sprinkled in the bottom of the furrow, using an appropriate measure giving the required quantity for each furrow length. The next furrow slice covered up the fumigant.

This method of application is not ideal but it is useful for experimental purposes with grasslands as it ensures even distribution. The furrow slice should be almost completely inverted and then pressed over by rolling so that the grass roots containing most of the wireworms are in close proximity to the fumigant. In the present instance the rolling was omitted, nevertheless the results were encouraging and suggestive for future work.

(2) *Wireworm counts and conclusions*

Before fumigation.

Late in March 1935, before treatment, three soil samples were taken at random from each half-plot, 150 samples in all, and examined separately for wireworms. Each sample was 9 in. square and taken to a depth of about 5 in.

The number of wireworms obtained in each sample is shown in

Table III

Knott Wood experiment. Wireworm count: after fumigation

Treat-ment	Serial no. of plot	A	B	C	Totals A	Totals B	Totals C	Totals Per plot
P	103	0,0	1,2	1,2	0	3	3	6
O	104	0,0	0,1	0,2	0	1	2	3
N	105	1,4	7,6	8,3	5	13	11	29
K	106	3,0	0,0	2,3	3	0	5	8
M	107	1,1	4,8	0,3	2	12	3	17
							Block total 63	
M	108	0,0	4,3	1,0	0	7	1	8
K	109	1,4	1,1	3,3	5	2	6	13
O	110	1,1	7,3	2,4	2	10	6	18
N	111	0,2	4,2	2,2	2	6	4	12
P	112	1,0	3,1	9,2	1	4	11	16
							Block total 67	
O	113	2,3	4,3	1,3	4	7	4	15
M	114	1,2	1,2	3,3	4	3	6	13
K	115	0,0	1,4	1,1	0	5	2	7
P	116	2,0	1,1	3,3	2	2	6	10
N	117	1,4	15,3	4,1	5	18	5	28
							Block total 73	
N	118	1,5	4,4	0,0	6	8	0	14
P	119	1,2	1,1	0,6	3	2	6	11
M	120	0,3	4,0	4,2	3	4	6	13
O	121	1,2	3,6	2,8	3	9	10	22
K	122	1,0	1,2	0,3	1	3	3	7
							Block total 67	
K	123	1,3	1,0	2,0	4	1	2	7
N	124	6,4	3,3	5,5	10	6	10	26
P	125	0,6	6,4	2,6	6	10	8	24
M	126	1,1	2,1	7,2	2	3	9	14
O	127	1,2	3,2	5,7	3	5	12	20
							Block total 91	

Per plot

General mean = 14·44.
Experimental error = ±7·185 = 49·8 % G.M.
Sampling error = ±4·87 = 34 % G.M.

Two samples were taken in each third of each plot, the sections being denoted in the table by A, B, C.

Table I, from which it is seen that there is some evidence of a gradient of infestation in the direction of the narrow side of the field. With few exceptions the north, that side away from the wood, was more heavily infested than the south side. The sampling error was 6·06 or 25 % and the experimental error 6·99 or 29 %. Thus the experimental error is almost entirely accounted for by the error in sampling.

After fumigation.

Owing to continuous rain delaying the second sampling, this was commenced only on 24 June, 9 weeks after the application of the fumigants, during very hot weather, and finished on 4 July. Two soil samples were taken in each third of every plot. Samples were taken across the beet rows and included at least one beet plant. In addition one plot in each block was sampled in between the rows. The number of wireworms per sample is shown in Table III.

Table IV shows the number per plot both before and after fumigation and Table IV A gives a comparison between the first and second counts calculated to numbers per square yard. Chlordinitrobenzene showed a reduction in numbers of 15 %, "*M*" and chlorpicrin 45 %, and "*K*"

Table IV

Knott Wood experiment. Wireworm counts: before and after fumigation

Totals of six soil samples (9 × 9 × 5 in.) per plot. Before above—After below.

	Treatments					
Block	Chlorpicrin	Chlordinitrobenzene	"*K*"	"*M*"	No fumigant	Block totals
I	6	20	34	19	6	85
	6	29	8	17	3	63
II	26	25	20	15	34	120
	16	12	13	8	18	67
III	33	22	24	17	20	116
	10	28	7	12	16	73
IV	24	39	32	36	24	155
	11	14	7	13	22	67
V	35	22	26	29	19	131
	24	26	7	14	20	91
Treatment	124	128	136	116	103	—
Totals	67	109	42	63	79	—

Standard errors per plot

Sampling	Experimental
First count ±6·06 = 25 %	±7 = 29 %
Second count ±4·87 = 34 %	±7·185 = 50 %

Table IV A

Knott Wood experiment. Summary of results: before and after fumigation

Mean number of wireworms per sq. yd. 5 in. deep

	No fumigant	Chlordinitrobenzene	Chlorpicrin	"*M*"	"*K*"	Mean	S.E.
Before fumigation	55	68	66	62	72	65	±8·34
After fumigation	42	58	36	34	22	38	±8·59
Actual difference	13	10	30	28	50	27	
Percentage drop	23	15	45	45	69	42	

69 %. On the untreated controls the density of the population had dropped 23 %, from 55 to 42 per sq. yd., due to seasonal changes, such as a possible movement of the wireworms to a greater depth to escape the heat, early pupation or loss by the activity of birds.

As the numbers of wireworms were so much lower, both the experimental error and sampling error per cent were higher with the second count than they were with the count before treatment. The sampling error was 34 % and the experimental error 50 %. A comparison of the numbers of wireworms at the beet with those found in the soil samples taken in between the rows is given below:

Total of six soil samples

Plot	In row	Between row	Difference −	Difference +
127 (*O*)	20	13	7	
112 (*P*)	16	9	7	
117 (*N*)	28	12	16	
122 (*K*)	7	11		4
107 (*M*)	14	14	0	0
	85	59	−26	

Total 144

Thus 59 % of the wireworms were found near the beet and 41 % in between the rows, some slight evidence that the wireworm population has been attracted to the beet. The negative result with the "*K*" plot might be due to the very poor growth of beet coupled with the low numbers of wireworms present.

The analysis of variance on wireworm counts showed that the effects of the treatments on the number of wireworms were not significant, with such a high experimental error (50 %), but that the differences with the "*K*" plots were nearly so. There was no apparent relation between the

numbers of wireworms per plot at the first and second counts after allowing for possible treatment effects.

Figs. 2 and 3 indicate graphically the changes in wireworm population after fumigation.

Effects of fumigants on the crop.

The beet was sown only 5 days after the fumigation of the soil. This resulted in a stunting of the growth of the plants on the plots treated with "*K*" but the other fumigants apparently had no harmful effects. It was important to see how long this deleterious action of "*K*" persisted. On 26 July the soil on the "*K*" plots still smelt strongly of the mixture. For the sake of comparison samples of soil were taken from all the fumigated plots and from the controls, and three pots were filled with the well-mixed soil from each treatment. Oats were planted in

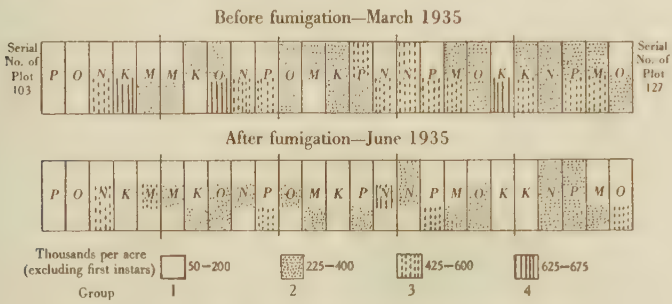


Fig. 2. Knott Wood pastures experiment, wireworm counts.

each pot and made good growth, ultimately reaching maturity. There was no essential difference between the oats grown in the treated soil and those grown in the untreated soil. Thus it would appear that the fumigants applied on 26 April had no longer any toxic effect on the plants 3 months later, even though in the case of "*K*" the soil still smelt strongly of the material.

Table V gives a summary of the yields of roots, tops and sugar obtained. The yield of roots with "*M*" was significantly higher than that with chlorpicrin or chlordinitrobenzene, the latter yields not being significantly different from the yield with no fumigant. The response to "*M*" may be an effect of nitrogen, the dressing being equivalent to 87 lb. nitrogen per acre.

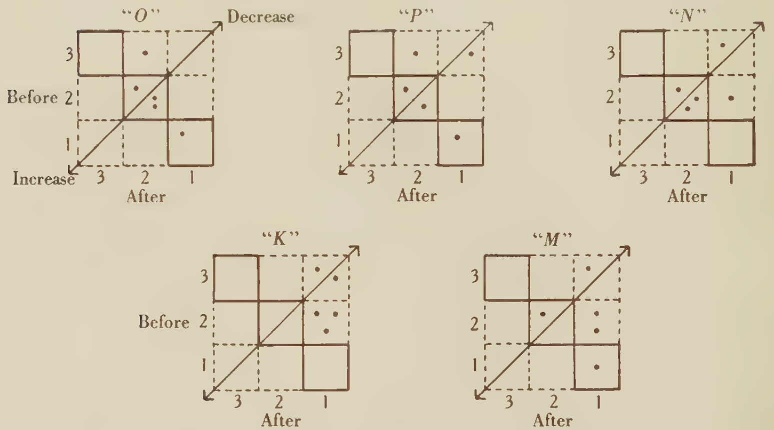
"*M*" and chlordinitrobenzene significantly increased the yields of tops, the increases not being significantly different. There were no significant effects on sugar percentage, apart from the reduction due to "*K*".

Table V

Knott Wood experiment. Beet crop. Summary of results

	No fumigant	Chlordi-nitroben-zene	Chlor-picrin	"*M*"	"*K*"	Mean	Standard error (treatment means)
Roots (washed), tons per acre	8·90	9·54	9·11	10·91	3·64	8·42	±0·426*
Tops, tons per acre	10·44	12·57	10·22	13·67	5·04	10·39	±0·519*
Sugar %	16·16	15·92	16·16	16·24	15·22	15·94	±0·130
Total sugar, cwt. per acre	28·8	30·4	29·4	35·4	11·1	27·0	—
Plant number, thousands per acre	28·6	29·0	29·4	30·0	26·3	28·7	±0·528

* These standard errors are not applicable to the "*K*" treatment.



Group	1	2	3
Thousands per acre	50–200	225–400	425–600

Fig. 3. Knott Wood pastures experiment. Change in distribution of wireworm population before and after fumigation.

The figure indicates graphically the changes in population estimated from samples taken before and after fumigation. The vertical scale is the population group before fumigation, and the horizontal scale the group after fumigation.

The heavily lined squares are areas of no change. Each dot represents one plot. Dots above the heavily lined squares represent plots which showed a decrease in population after fumigation, and dots below them represent plots which showed an increase.

Thus with "*O*", four plots remain in the same population group and one plot changes from group 3 (425–600 thousands) to group 2 (225–400 thousands), whereas with "*K*" three plots change from group 2 before fumigation to group 1 (50–200 thousands) afterwards, and two plots from group 3 to group 1.

The average change, as a percentage on the original count, is as follows: "*O*" −23, "*P*" −45, "*N*" −15, "*K*" −69, "*M*" −45.

"*K*" gave low yields and a low sugar percentage. There is no doubt that this was because it was applied too near the sowing date.

No relation was found between the yields of roots and the numbers of wireworms at the second count, after eliminating treatment effects.

Residual effects.

No further treatment was given in 1936, but barley was drilled on 20 March. Four soil samples (6×6 in.) were taken per plot, two samples in each half-plot, in June 1936, one year after the second soil sampling. The total number of wireworms obtained from the five plots for each treatment was as follows:

"*O*"	29
Chlorpicrin	23
Chlordinitrobenzene	35
"*K*"	6
"*M*"	31

The figures for separate plots are given in Table VI. Thus the fewest wireworms were again found on the "*K*" plots.

Table VI

Knott Wood experiment. Residual effect

Number of wireworms per plot June 1936. Total of 4 samples ($6 \times 6 \times 5$ in.)

					Rows
P 3	*O* 2	*N* 5	*K* 1	*M* 4	15
M 6	*K* 0	*O* 6	*M* 4	*P* 4	20
O 4	*M* 9	*K* 1	*P* 6	*N* 5	25
N 17	*P* 8	*M* 8	*O* 9	*K* 0	42
K 4	*N* 4	*P* 2	*M* 4	*O* 8	22
Columns 34	23	22	24	21	124

G.M. = 4·96. Sampling error 48 %. Experimental error = ±2·976 = 60 %.

The mean stand of plants per 4 m. length was counted in May and varied from 140 on the untreated plots to 197 on the plots which had been treated with "*K*" in 1935. Thus the plots with the biggest reduction of the wireworm population gave the best stand of barley. However, these differences in the plant numbers were not reflected in the final yield of grain. The controls yielded an average of 18·1 cwt. per acre, and

the treated plots varied from 19·5 cwt. with "*K*" to 21·1 cwt. with chlordinitrobenzene. Either the general infestation was not high enough to cause material damage or the plants had made extra growth to compensate for damage done. The crop of barley was a moderately good one for this season which was not particularly favourable to cereals. It is suggested that the high yield from the chlordinitrobenzene plots might be due to delayed nitrogen effect, the chlorine having inhibited the breakdown of the compound by bacterial action in the year of application.

Residual effect, summary of results

Mean number of wireworms per sq. yd. to 5 in. deep

No fumigant	Chlordinitro-benzene	Chlorpicrin	"*M*"	"*K*"	Mean	S.E.
52	63	41	56	11	45	±12

The mean number of wireworms found is 45 per sq. yd. compared with 38 per sq. yd. in June 1935. This increase is more apparent than real and is attributed to the improvement in the technique for separating the wireworms, whereby the last count includes the earlier instars which had been missed in the first and second counts.

V. High Field experiment I

(1) *Lay-out and design of experiment*

For this experiment an area of old park land was selected in High Field, Rothamsted, to break up for a crop of barley. The object of the experiment was to try out "*S*" and chlorpicrin, these being two of the most promising fumigants from the previous trial. In this experiment, it was decided to increase the replication from five to ten and reduce the samples from six to four. The plots were $\frac{1}{70}$ acre in area (25·25 × 25 ft.) and arranged in ten randomized blocks of three plots each. (For plan see Fig. 4.)

There were two treatments and a control.

	Rate per acre
(*S*)*	800 lb.
Chlorpicrin (*C*)†	245 lb.
No fumigant	

* A similar mixture to "*K*" used in Experiment 1 but containing a higher proportion of *o*-dichlorbenzene.

† In the form of pellets containing 25% chlorpicrin.

The fumigants were applied in the same fashion as in the Knott Wood Experiment by sprinkling an appropriate quantity at the bottom of each plough furrow.

(2) *Wireworm counts and conclusions*

Before fumigation.

At the end of January 1936 two soil samples (9 × 9 × 5 in.) were taken from each half-plot, a total of 120 samples. The numbers of wireworms found in each sample are shown in Table VII. The total number of wireworms found was 2516, an average of 21 per sample. This is five times the number found in the preliminary examination in the Knott Wood experiment, the difference being mostly due to an actually higher infestation, but partly to the improvement in the technique which made it possible to discover the smaller wireworms which were missed by the crude methods used at first.

Table VII

High Field I experiment. Wireworm counts: before fumigation

Two soil samples per half-plot. Size of samples: 9 × 9 × 5 in.

Plot totals thus $\boxed{99}$

Plots 1–15

Block	Chlorpicrin	"S"	No fumigant	Block totals
I	20 6 $\boxed{74}$ 36 12	21 18 $\boxed{78}$ 18 21	36 15 $\boxed{99}$ 30 18	251
II	21 13 $\boxed{71}$ 23 14	20 19 $\boxed{79}$ 20 20	14 12 $\boxed{72}$ 28 18	222
III	23 8 $\boxed{65}$ 21 13	20 34 $\boxed{96}$ 18 24	10 7 $\boxed{60}$ 13 30	221
IV	18 20 $\boxed{95}$ 27 30	32 30 $\boxed{92}$ 20 10	31 6 $\boxed{78}$ 25 16	265
V	28 15 $\boxed{84}$ 20 21	58 39 $\boxed{137}$ 21 19	19 25 $\boxed{73}$ 17 12	294
Treatment totals	389	482	382	1253

G.M. = 83·87. Sampling error = 18·7 %. Experimental error ± 17·75 = 21·2 %.

Table VII (*cont.*)

Plots 16–30

Block	Chlorpicrin	"*S*"	No fumigant	Block totals
VI	25 16 $\boxed{95}$ 36 18	34 12 $\boxed{105}$ 24 35	14 26 $\boxed{69}$ 18 11	269
VII	13 19 $\boxed{66}$ 9 25	28 19 $\boxed{75}$ 14 14	24 28 $\boxed{100}$ 18 30	241
VIII	21 7 $\boxed{54}$ 7 19	16 19 $\boxed{118}$ 18 65	22 7 $\boxed{82}$ 26 27	254
IX	11 21 $\boxed{66}$ 16 18	19 26 $\boxed{86}$ 20 21	33 33 $\boxed{105}$ 18 21	257
X	12 16 $\boxed{74}$ 26 20	15 21 $\boxed{98}$ 48 14	4 32 $\boxed{69}$ 26 7	241
Treatment totals	355	482	425	1262

The larger number of wireworms and greater replication gave lower sampling and experimental errors per cent: 18·7 and 21·2 respectively.

After fumigation.

Owing to various adverse circumstances, the interval between the fumigation and the second sampling was again longer than desirable, being a minimum of 12 weeks.

The second sampling commenced on 30 April and extended to 16 May, two blocks generally being sampled in a day. The size of the samples was $6 \times 6 \times 5$ in. and two were taken in each half-plot.

Table VIII gives the numbers of wireworms found in each sample. The block totals varied from 51 to 113 with a mean of 75, a block total representing the number of wireworms found in twelve soil samples.

The treatment totals for ten plots are as follows: "*S*", 165, chlorpicrin, 221 and no fumigant, 363. The means are: "*S*", 16·5, chlorpicrin, 22·1 and no fumigant, 36·3, with a general mean of 24·97.

Table IX compares the plot totals before and after fumigation. Table IX A shows that the extension of the sampling period over 6 weeks has not affected the results.

Table IX B, which summarizes the wireworm counts before and after fumigation, shows that the average density of population on the untreated plots has not materially changed, being 323 per sq. yd. before fumigation and 327 after fumigation, an increase of only 1·2 %. This is in contrast to the Knott Wood experiment where a drop of 23 % was found. The period covered by the two counts on Knott Wood was March–July, whereas on High Field I it was January–May. A possible explanation is a downward movement of the wireworms in June to below the depth of sampling, as mentioned above, and loss by pupation. "*S*" has reduced the population by 61·2 % and chlorpicrin by 33·2 %. Both these

Table VIII

High Field I experiment. Wireworm count: after fumigation

Two samples per half-plot. Size of samples: 6 × 6 × 5 in.
Total per plot in central square

Plots 1–15

Blocks	Chlorpicrin	"*S*"	"*O*"	Block totals
I	5 4 $\boxed{16}$ 5 2	5 5 $\boxed{13}$ 1 2	12 20 $\boxed{48}$ 8 8	77
II	0 9 $\boxed{15}$ 3 3	6 4 $\boxed{19}$ 5 4	7 4 $\boxed{20}$ 4 5	54
III	4 4 $\boxed{20}$ 3 9	2 9 $\boxed{21}$ 3 7	9 6 $\boxed{33}$ 7 11	74
IV	7 3 $\boxed{27}$ 5 12	6 4 $\boxed{22}$ 8 4	12 22 $\boxed{64}$ 17 13	113
V	4 9 $\boxed{27}$ 8 6	2 9 $\boxed{21}$ 7 3	7 8 $\boxed{29}$ 5 9	77
Treatment totals	105	96	194	395

G.M. = 24·97. Sampling error = 23·8 %. Experimental error ± 8·43 = 33·8 %.

Table VIII (*cont.*)

Plots 16–30

Blocks	Chlorpicrin	"S"	"O"	Block totals
VI	8 15 $\boxed{32}$ 5 4	1 5 $\boxed{8}$ 0 2	14 7 $\boxed{45}$ 16 8	85
VII	4 4 $\boxed{14}$ 3 3	5 4 $\boxed{21}$ 6 6	7 9 $\boxed{28}$ 2 10	63
VIII	3 2 $\boxed{22}$ 13 4	6 3 $\boxed{14}$ 3 2	4 5 $\boxed{35}$ 18 8	71
IX	11 12 $\boxed{31}$ 3 5	3 5 $\boxed{18}$ 5 5	10 12 $\boxed{35}$ 8 5	84
X	7 6 $\boxed{17}$ 3 1	4 4 $\boxed{8}$ 0 0	6 9 $\boxed{26}$ 3 8	51
Treatment totals	116	69	169	354
Grand totals	221	165	363	749

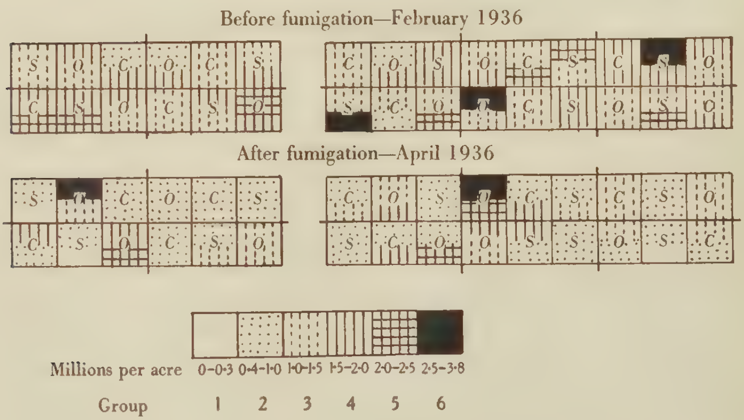


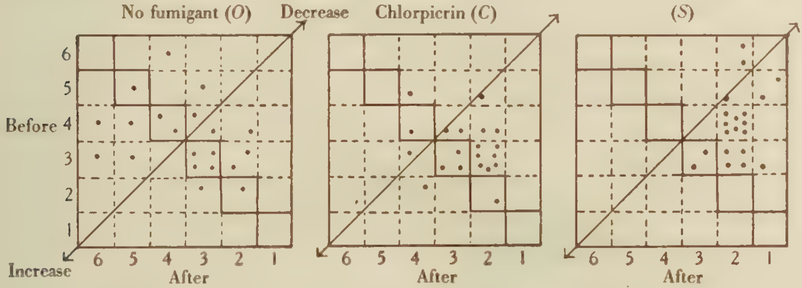
Fig. 4. High Field I, wireworm counts. The variation in shading indicates the population densities per half-plot.

differences are significant. Figs. 4 and 5 show graphically the changes after fumigation.

The sampling error was 23·8 % of the general mean, not much greater than that of the sampling before fumigation in spite of the smaller sampling unit. The experimental error was 33·8 %, higher than that of the preliminary sampling. As in the first experiment, there was no relation between the numbers of wireworm per plot at the first and second counts after allowing for treatment effects. As far as the wireworm counts are concerned this experiment was quite satisfactory but it was not so successful with the crop.

Effect of fumigants on crop.

Soon after the barley was planted it was noticed that the birds were very busy on the plots and paper streamers were fixed in order to frighten



Group	1	2	3	4	5	6
Millions per acre	0–0·3	0·4–1	1–1·5	1·5–2	2–2·5	2·5–3·8

Fig. 5. High Field I. Change in distribution of wireworm population before and after fumigation.

The figure indicates graphically the changes in population estimated from samples taken before and after fumigation. The vertical scale is the population group before fumigation, and the horizontal scale the group after fumigation. The heavily lined squares are areas of no change. Each dot represents one half-plot. Dots above the heavily lined squares represent plots which showed a decrease in population after fumigation, and dots below them represent plots which showed an increase.

The diagram clearly shows the big reduction in population brought about by the use of chlorpicrin and the still bigger reduction by the fumigant "*S*". With no fumigant there are eight half-plots that show no change in the population group, five increases and seven small decreases. With chlorpicrin there are five half-plots showing no change, two small increases, and twelve decreases, one of which is from group 5 (2–2·5 million) to group 2 (0·4–1 million) and two from group 4 (1·5–2 million) also to group 2. With "*S*" only two half-plots remain in the same group, there are eighteen decreases, two of which are from group 6 (2·5–3·8 million) to group 2, two from group 5 to group 1 (below 0·3 million), one from group 5 to group 2, and eight from group 4 to group 2. The average change as a percentage on the count before fumigation is "No fumigant" +1·2, Chlorpicrin −33·2, "*S*" −62·2.

Table IX

High Field I experiment. Wireworm counts: before and after fumigation

Plot totals: first count above, second count below.*
Total of four samples (9 × 9 × 5 in.) per plot

Plots 1–15

Blocks	Chlorpicrin	"*S*"	No fumigant	Block totals
I	74	78	99	251
	36	29	109	174
II	71	79	72	222
	34	43	45	122
III	65	96	60	221
	45	48	75	168
IV	95	92	78	265
	61	50	144	255
V	84	137	73	294
	61	48	64	173
Treatment totals:				
Before	389	482	382	
After	237	218	437	

Plots 16–30

Blocks	Chlorpicrin	"*S*"	No fumigant	Block totals
VI	95	105	69	269
	72	18	112	202
VII	66	76	100	242
	32	48	64	144
VIII	54	118	82	254
	50	33	78	161
IX	66	86	105	257
	70	40	79	189
X	74	98	69	241
	39	18	59	116
Treatment totals:				
Before	355	483	425	
After	263	157	393	

* Actual size of sample at second count 6 × 6 × 5 in. Figures calculated to 9 × 9 in. for comparison with first count.

Table IXA

High Field I experiment. Wireworm counts: after fumigation

Dates of sampling and block totals

Plots no.	Date of sampling	Wireworms in blocks of three plots
1, 2, 3	30 April	77
4, 5, 6	2 May	54
19, 20, 21	2 ,,	63
7, 8, 9	8 ,,	74
16, 17, 18	8 ,,	85
10, 11, 12	12 ,,	113
25, 26, 27	12 ,,	84
22, 23, 24	14 ,,	71
13, 14, 15	16 ,,	77
28, 29, 30	16 ,,	51

Table IXB

High Field I experiment. Summary of results: before and after fumigation

Mean no. of wireworms per sq. yd. (5 in. deep)

	No fumigant	Chlor-picrin	"*S*"	Mean	S.E.
Before fumigation	323	298	386	335	±41
After fumigation	327	199	150	225	±44
Difference:					
Actual	+4	−99	−236	−110	
% on original count	1·2	−33·2	−62·2	−32·8	

them away. There is no doubt that they removed a large number of the germinating seedlings and probably some of the wireworms as well. It was not possible to observe whether they concentrated on some plots more than others, but fumigants used may have had a deterrent effect on the birds which would then tend to gather more from the untreated plots.

A stand count was taken on 13 May. The mean number of plants per 4 m. length varied from 52 on the "*S*" plots to 85 on the chlorpicrin plots, with the controls occupying an intermediate position with 68. The reduction in stand on the "*S*" plots was not quite significant but the increase in the stand on the chlorpicrin plots was significant.

There was an interval of 6 weeks between the application of the fumigants and drilling the barley. Evidently this interval was not long enough to dissipate the phytocidal action of the "*S*" with the large quantity used, 8 cwt. compared with 5 cwt. used in Knott Wood. In view of the poor stand it was decided that no further information would be obtained by allowing the crop to mature, so the land was harrowed

and sugar-beet planted. Before this, the second soil sampling had been completed. The barley should have been grubbed out immediately, but unfortunately a spell of very wet weather intervened and, before the barley was pulled, it had grown to the flowering stage and choked out the sugar-beet. Thus the experiment finished with no crop at all.

Residual effect.

No further treatment was given in 1937 but barley was sown once again. A third wireworm count was not made as it was considered that the time available would be better employed by starting a new experiment.

Stand count. The stand of barley was poor and very similar to that of 1936, the means varying from 55 plants per 4 m. on the untreated plots to 66 on the "*S*" plots and 69 on the chlorpicrin plots. The average increase on the fumigated plots was significant, but there was no indication of any difference between the treatments.

Yields. The final yield of barley was very poor; this may have been due partly to the fact that no fertilizers were used and partly to damage by wireworms. The controls yielded an average of 10·1 cwt.: "*S*", 9·6 cwt. and chlorpicrin, 12·3 cwt. of grain per acre. The response to chlorpicrin was significant. This increase may be a result of the reduction in the number of wireworms, but on the other hand the possibility of a delayed nitrogen effect due to the breakdown of the chlorpicrin cannot be ignored.

VI. High Field experiment II

(1) *Lay-out and design of experiment*

The object of the experiment was to test the effect of various materials, frequently recommended as efficacious, on wireworm infestation in old grassland which was ploughed up and summer fallowed for winter corn.

There were 48 plots of $\frac{1}{200}$ acre in randomized blocks.

The following treatments were given:

No treatment.

Lime: 34 cwt. per acre.

Tar and lime (33 % tar): 51 cwt. per acre.

Ammonium carbonate: 70 lb. per acre (equivalent in nitrogen content to the tar).

Superphosphate: 6 cwt. per acre.

Naphthalene (crude): 10 cwt. per acre.

Fig. 6 shows the plan for the experiment.

The fumigants were applied at the bottom of the plough furrows on 9 April 1937. On 11 April the land was rolled to complete the inversion of the furrow slice and consolidate the soil.

(2) *Wireworm counts and conclusions*

Soil sampling before treatment.

Two soil samples (6 × 6 × 6 in.) per plot were taken on 23 and 24 March, a total of 96 samples, 16 days before fumigants were applied. The soil was very wet after weeks of persistent rainfall which made the subsequent examination very troublesome.

The number of wireworms per soil sample and per plot are shown in Table X. The figures range from 1 to 19 per sample with an average of 7·2, and from 4 to 32 per plot with an average of 15·38. The sampling error and experimental error are practically the same, 36·8 and 35·6 %

Table X

High Field II experiment. Wireworm count: before treatment

Two soil samples per plot (6 × 6 × 6 in.). Plot totals in squares

Blocks	Treatments																		Block totals
	O			Lime			Tar + lime			Am. carb.			Super.			Naphth.			
I	13	20	7	2	7	5	15	20	5	9	21	12	7	16	9	10	22	12	106
II	13	20	7	9	14	5	10	21	11	6	16	10	7	18	11	5	12	7	101
III	14	25	11	7	19	12	5	9	4	3	7	4	7	8	1	11	20	9	88
IV	8	9	1	5	8	3	5	21	16	12	18	6	8	10	2	6	13	7	79
V	2	4	2	4	13	9	3	10	7	7	8	1	7	13	6	6	10	4	58
VI	7	18	11	11	13	2	7	16	9	8	13	5	4	16	12	4	22	18	98
VII	15	19	4	8	12	4	9	16	7	9	14	5	4	7	3	13	32	19	100
VIII	11	23	12	3	14	11	10	16	6	10	16	6	12	16	4	15	23	8	108
Treatment totals	138			100			129			113			104			154			738

G.M. = 15·38. Sampling error ± 5·65 = 36·8 %. Experimental error ± 5·48 = 35·6 %.

respectively, i.e. the inter-plot variation is no more than the variation between individual soil samples. The two soil samples only represented 0·23 % of the total area of the plot, this small proportion being a contributory cause of the high sampling error.

The average density of the wireworm population was 277 per sq. yd., which is considerably less than the 335 per sq. yd. found on High Field in January 1936.

Soil sampling after treatment.

Again two 6 in. samples were taken from each plot. The sampling and examination commenced some weeks after the application of the treatments extending from 1 to 24 June. Details of the numbers of wireworms found are shown in Table XI and a comparison of the counts before and after treatment are shown in Tables XII and XII A. The figures for individual samples ranged from 2 to 16 with a mean of 6·25. The figures per plot were 5–23 with a mean of 12·5. The sampling error

Table XI

High Field II experiment. Wireworm count: after treatment

Two soil samples per plot (6 × 6 × 6 in.). Plot totals in squares

Block	Treatments																		Block totals
	No treatment			Lime			Tar + lime			Am. carb.			Super.			Naphth.			
I	8	$\boxed{18}$	10	8	$\boxed{19}$	11	10	$\boxed{14}$	4	10	$\boxed{17}$	7	9	$\boxed{16}$	7	3	$\boxed{10}$	7	94
II	4	$\boxed{18}$	14	5	$\boxed{8}$	3	8	$\boxed{13}$	5	5	$\boxed{7}$	2	5	$\boxed{11}$	6	2	$\boxed{6}$	4	63
III	14	$\boxed{21}$	7	8	$\boxed{13}$	5	4	$\boxed{11}$	7	9	$\boxed{15}$	6	8	$\boxed{12}$	4	3	$\boxed{5}$	2	77
IV	9	$\boxed{20}$	11	7	$\boxed{15}$	8	2	$\boxed{10}$	8	8	$\boxed{12}$	4	16	$\boxed{20}$	4	3	$\boxed{7}$	4	84
V	10	$\boxed{15}$	5	5	$\boxed{9}$	4	5	$\boxed{9}$	4	3	$\boxed{9}$	6	3	$\boxed{11}$	8	3	$\boxed{5}$	2	58
VI	5	$\boxed{8}$	3	16	$\boxed{23}$	7	4	$\boxed{8}$	4	3	$\boxed{7}$	4	7	$\boxed{11}$	4	4	$\boxed{7}$	3	64
VII	5	$\boxed{16}$	11	7	$\boxed{15}$	8	3	$\boxed{6}$	3	2	$\boxed{15}$	13	4	$\boxed{15}$	11	7	$\boxed{11}$	4	78
VIII	6	$\boxed{10}$	4	10	$\boxed{15}$	5	9	$\boxed{21}$	12	5	$\boxed{8}$	3	11	$\boxed{17}$	6	3	$\boxed{11}$	8	82
Treatment totals		126			117			92			90			113			62		600

G.M. = 12·5. Sampling error ±4·45 = 35·6 %. Experimental error ±3·90 = 31·2 %.

was 35·6 %, accounting for the whole of the experimental error which is 31·2 %.

Table XII

High Field II experiment. Wireworm counts: before and after treatment

Total of two soil samples (6 × 6 × 6 in.) per plot. First count above, second count below

Blocks	No treatment	Lime	Tar + lime	Am. carb.	Super.	Naphth.	Block totals	Diff.
	Treatments							
I	20	7	20	21	16	22	106	
	18	19	14	17	16	10	94	−12
II	20	14	21	16	18	12	101	
	18	8	13	7	11	6	63	−38
III	25	19	9	7	8	20	88	
	21	13	11	15	12	5	77	−11
IV	9	8	21	18	10	13	79	
	20	15	10	12	20	7	84	+ 5
V	4	13	10	8	13	10	58	
	15	9	9	9	11	5	58	0
VI	18	13	16	13	16	22	98	
	8	23	8	7	11	7	64	−34
VII	19	12	16	14	7	32	100	
	16	15	6	15	15	11	78	−22
VIII	23	14	16	16	16	23	108	
	10	15	21	8	17	11	82	−26
Treatment totals:								
Before	138	100	129	113	104	154		
After	126	117	92	90	113	62		
Diff.	−12	+17	−37	−43	+9	−92		

Table XII A

High Field II experiment. Summary of results: before and after treatment

Mean no. of wireworms per sq. yd. (6 in. deep)

	No treatment	Lime	Tar + lime	Am. carb.	Super.	Naphth.	Mean	S.E.
Before treatment	310	225	290	254	234	346	277	±40
After treatment	283	263	207	202	254	139	225	±29
Difference:								
Actual	−27	+38	−83	−52	+20	−207	−52	
% on original count	− 8·7	+17	−28·6	−20·4	+ 8·6	− 59·8	−18·8	

It will be seen from the treatment totals given in Table XI that all the treated plots have fewer wireworms than the untreated plots. *Taking*

the "no treatment" number as a datum and calculating the differences as a percentage on this, the following figures are obtained:

		Differences	
		Actual	Percentage
No treatment	126	—	—
Lime	117	− 9	− 7·2
Superphosphate	113	−13	−10·3
Tar and lime	92	−34	−27
Ammonium carbonate	90	−36	−28·6
Naphthalene	62	−64	−50·5

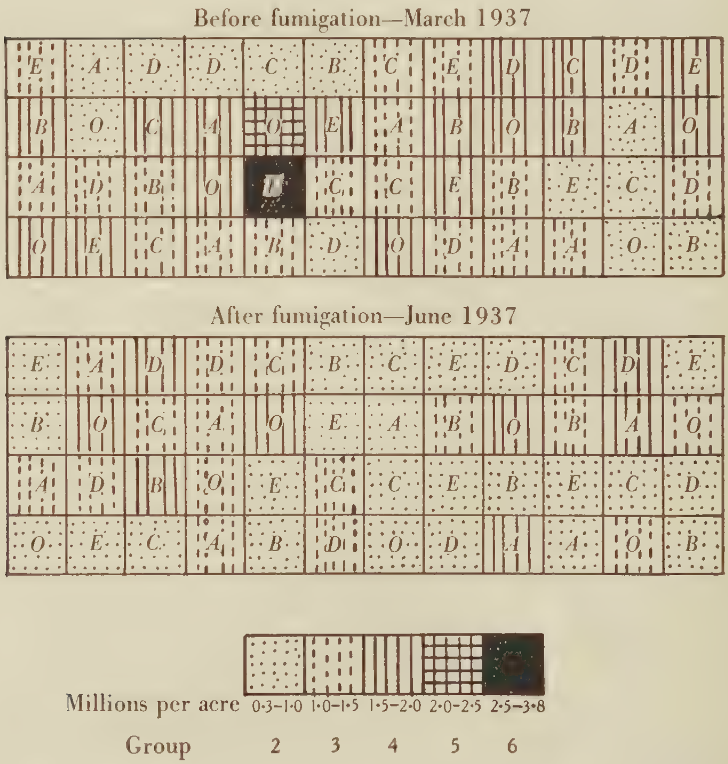


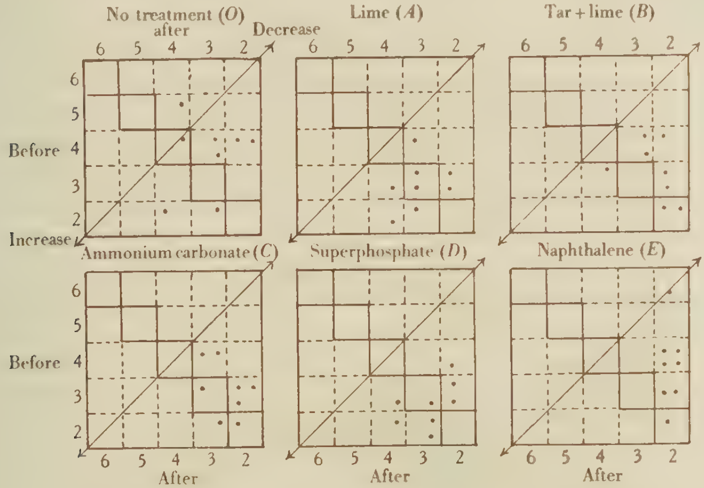
Fig. 6. High Field II, wireworm count. The variation in shading indicates the population densities per plot.

The analysis of variance showed that these differences were significant in the case of tar and lime, ammonium carbonate and naphthalene, but the differences between the latter and the two former is not quite

significant. Figs. 6 and 7 indicate graphically the changes after treatment.

Comparing the counts before and after treatment (Table XII A).

It is seen that the mean density of the wireworm population had fallen from 277 to 225 per sq. yd. The untreated plots have dropped by



Group	1	2	3	4	5	6
Millions per acre	0–0·3	0·3–1	1–1·5	1·5–2	2–2·5	2·5–3·8

Fig. 7. High Field II. Change in distribution of wireworm population, before and after treatment.

The figure indicates graphically the change in population estimated from samples taken before and after fumigation. The vertical scale is the population group before fumigation and the horizontal scale the group after fumigation. The heavily lined squares are areas of no change. Each dot represents one plot. Dots above the heavily lined squares represent plots which showed a decrease in population after fumigation, and dots below them represent plots which showed an increase. For example, the untreated plots show five decreases, one unchanged and two increases, whereas the naphthalene plots show seven decreases of which one is from group 6 (2·5–3·8 million) to group 2 (0·3–1 million) and four are from group 4 (1·5–2 million) also to group 2.

The average change, as a percentage on the count before fumigation is as follows: no treatment −8·7, lime +17, tar + lime −28·6, ammonium carbonate −20·4, superphosphate +8·6, naphthalene −59·8.

8·7 %, ammonium carbonate by 20·4 %, tar and lime by 28·6 %, and naphthalene by 59·8 %, whereas the limed plots have increased by 17 % and superphosphate by 8·6 %. As far as the significant treatments are concerned these results are not materially different from the conclusions obtained from the consideration of the second count alone. The apparent

increases on the lime and superphosphate plots are small and of no real significance. The failure of lime to reduce the number of wireworms is interesting in view of its frequent recommendation amongst farmers. Superphosphate has often been mentioned as a dressing for land infested with wireworms, but a direct toxic effect would be unlikely. Any value it might have would depend on its stimulating action on root growth, thus encouraging the crop to grow away quickly from the attack. Although the reduction in the wireworm population produced by the fumigants (even the 59·8 % with naphthalene) is too low to be of much economic value, yet the figures are useful as indicating possible means of control. More adequate control could be expected, given a finer physical condition of the fumigant, and a better method of application resulting in a more complete distribution throughout the soil mass. It will be necessary to find out how far the reduction in population by any treatment is due to a repellent action and how far it is actually lethal and also whether any repellent action is lateral or downwards. Experiments are being made to throw light on these points.

The reduction in numbers of 8·7 % between the first and second samplings on the control plots is less than that found in the Knott Wood experiment and more than on High Field I, thus:

	Time of sampling		Average difference in controls
	1st	2nd	%
Knott Wood	March	July	−23
High Field I	January	May	+ 1·2
High Field II	March	June	− 8·7

A number of the wireworms found in the second sampling of High Field II were in process of moulting, and some were actually pupating but not enough to affect the percentage drop. As already suggested, the reduction when the second sampling was done in June and the still bigger one when the sampling was done in July might well be due to aestivation but more critical experiments would be required to elucidate this point.

VII. Selective action of fumigants on wireworms of different ages

In any experiments on the control of wireworms it is important to know whether a particular treatment has any selective action on the various instars. Are the young larvae killed more easily than those nearly fully grown or is there any particular stage of growth when the

Table XIII

High Field I experiment. Wireworms grouped according to length: before and after fumigation

(*a*) Difference in individual groups according to treatments.

	No fumigant ("*O*")				Chlorpicrin ("*C*")				("*S*")			
			Diff.				Diff.				Diff.	
Group	Before	After	Actual	%	Before	After	Actual	%	Before	After	Actual	%
1 (<0·5 cm.)	101	95	− 6	− 5·9	76	65	− 11	−14·4	137	20	−117	−85·3
2 (0·6–0·9 cm.)	351	441	+90	+25·7	330	234	− 96	−29·1	451	191	−260	−57·5
3 (1·0–1·3 cm.)	153	144	− 9	− 5·9	165	61	−104	−63·0	190	79	−111	−58·3
4 (1·4–1·6 cm.)	138	90	− 48	−34·8	116	74	− 42	−36·2	134	45	− 89	−66·5
5 (1·7–2 cm.)	56	23	−33	−58·9	53	29	− 24	−45·3	45	29	− 16	−35·5
6 (>2 cm.)	8	—	− 8	—	4	9	+ 5	—	8	2	− 6	—
Treatment totals, 40 soil samples 9 × 9 in.	807	793*			744	472*			965	366*		

(*b*) General composition (as % of totals)

	All treatments together		Separate treatments					
			"*O*"		"*C*"		"*S*"	
Group	Before	After	Before	After	Before	After	Before	After
1	13	11	12	12	10	14	14	6
2	48	53	44	56	45	49	46	52
3	19	17	19	18	22	13	20	21
4	13	13	17	11	15	16	14	12
5	6	5	7	3	7	6	5	8
6	1	1	1	0	1	2	1	1
	100	100	100	100	100	100	100	100

* These totals are slightly below those shown in Table IX as some wireworms were mutilated and could not be measured.

insects are more susceptible than at other times? Unfortunately it is impossible to differentiate rapidly between different species or to identify the particular instars, but failing any better method the length of the larva gives some indication of its age. As mentioned on p. 348, the wireworms were measured and divided into six arbitrary groups and Table XIII shows the distribution of the wireworms according to these groups on High Field I. It will be seen that the average composition of the catch in April after fumigation is much the same as in January before fumigation, with a slight increase in group 2 from 48 to 53 % and a reduction in group 3 from 19 to 17 %, but an examination of the grouping according to treatments shows that the fumigants do appear to have had some effect on the distribution. However, in assessing the differential effect of chlorpicrin and "*S*" on the various sizes of wireworms, due allowances must be made for normal growth, i.e. moving up from one group to another as shown for instance in group 2 in the unfumigated plots. "*S*" appears to have been most severe on group 1 which is reduced by 58 % whereas chlorpicrin only reduced it by 14 %. In group 2, "*S*" has effected a reduction of 58 % whereas chlorpicrin has reduced it by 29 % only. Group 3 appears to have been affected similarly by both fumigants. In group 4, the reduction by chlorpicrin (36 %) is no higher than that of the unfumigated plots (35 %), whereas "*S*" has reduced this group by 67 %. The numbers in the other two groups are too small for any legitimate comparison.

Table XIV shows the distribution of the wireworms over the various groups in the High Field II experiment. The actual numbers are much smaller than in High Field I, as the soil samples were smaller, fewer per plot and fewer replicates.

The sampling before fumigation was done in April and that after fumigation in June and as with High Field I the general composition of the counts was much the same in both.

Comparison of the first count in High Field II with the first count in High Field I shows, however, marked differences in some of the groups. Thus group 1 comprises only 8 % of the wireworms in High Field II whereas it reached 13 % in High Field I. Group 2 is 39 % compared with the 47 % of High Field I, but in group 3 the positions are reversed High Field II having 28 % compared with only 19 % in High Field I.

Taking groups 2 and 3 together there is no difference, these accounting for 66 and 67 % respectively, so that the differences in the individual groups might be merely the results of the normal growth between the months of January and April. In that case, however, it would be expected

Table XIV

High Field II experiment. Wireworms grouped according to length: before and after fumigation

(*a*) Difference in individual groups according to treatments

	No fumigant ("*O*")				Lime ("*A*")				Tar + lime ("*B*")				Am. carb. ("*C*")			
			Diff.				Diff.				Diff.				Diff.	
Group	Before	After	Actual	%	Before	After	Actual	%	Before	After	Actual	%	Before	After	Actual	%
1	7	8	+ 1	+14·2	13	15	+ 2	+15·4	9	6	− 3	−33·3	9	11	+ 2	+22·2
2	41	47	+ 6	+14·6	37	41	+ 4	+10·8	50	22	−28	−56·0	46	25	−21	−45·5
3	48	39	− 9	−18·7	17	29	+12	+70·5	36	24	−12	−33·3	31	30	− 1	− 3·2
4	21	16	− 5	−23·8	18	19	+ 1	+ 5·6	17	24	+ 7	+41·2	13	11	− 2	−15·4
5	20	13	− 7	−35	11	15	+ 4	+36·4	11	11	0	—	12	13	+ 1	+ 8·33
6	0	0	—	—	2	0	− 2	—	2	1	− 1	—	2	2	0	—
Treatment totals, 16 soil samples	137	123	−14		98	119	+21		125	88	−37		113	92	−21	

	Superphosphate ("*D*")				Naphthalene ("*E*")			
			Diff.				Diff.	
Group	Before	After	Actual	%	Before	After	Actual	%
1	6	10	+ 4	+66·6	12	4	− 8	−66·6
2	32	46	+14	+43·7	73	23	−50	−68·5
3	30	20	−10	−33·3	39	11	−28	−17·5
4	17	18	+ 1	+ 5·9	14	7	− 7	−50
5	12	10	− 2	−16·7	9	15	+ 6	+66·6
6	—	—	—	—	1	2	+ 1	—
Treatment totals, 16 soil samples 6 × 6 in.	97	104	+ 7	—	148	62	−86	—

Note. These treatment totals are below those shown in Table XII as some of the wireworms were dead or mutilated and could not be measured.

(*b*) General composition (as % of total)

	All treatments together		Separate treatments											
			"*O*"		"*A*"		"*B*"		"*C*"		"*D*"		"*E*"	
Group	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	8	9	5·2	6·5	13·3	12·6	7·2	6·8	8·0	11·9	6·2	9·6	8·1	6·5
2	39	35	29·9	38·3	37·8	34·5	40·0	25·1	40·7	27·2	33·1	44·3	49·3	37·1
3	28	26	35·5	31·7	17·3	24·4	28·8	27·2	27·4	32·7	30·9	19·2	26·3	17·7
4	14	16	15·3	13·0	18·4	15·9	13·6	27·2	11·5	11·9	17·5	17·3	9·5	11·3
5	10	13	14·6	10·5	11·2	12·6	8·8	12·5	10·6	14·1	12·3	9·6	6·1	24·2
6	1	1	0·0	0·0	2·0	0·0	1·6	1·2	1·8	2·2	0·0	0·0	0·7	3·2
	100	100	100	100	100	100	100	100	100	100	100	100	100	100

that the composition of the first count in High Field II would agree with the second count of High Field I on the unfumigated plots, both being taken in April, but as the figures in Table XIV B ("*O*", before) and in Table XIII B ("*O*", after) show no such agreement, it must be concluded that the differences are probably due to environmental factors.

Examining the distribution according to treatments, it will be noted that, on the untreated plots, there was an increase of the first two groups by about 14 % and a decrease in the other groups of from 18 to 35 %. No wireworms were found in group 6 either in April or June, but in June some of group 5 were pupating and in all groups individuals were moulting. Thus a moving up in the groups was actually taking place at the time of the second count and the pupating would produce a drop in groups 5 and 6. There was a general increase in all the groups on the limed plots, particularly in group 3. Tar and lime showed a good reduction in groups 1, 2, and 3 of which group 2 was the most (56 %), an increase in group 4 and no change in group 5. Ammonium carbonate gave a big reduction in group 2 (46 %) but no significant change otherwise. Superphosphate showed an increase in groups 1 and 2, a drop of 33 % in group 3 and no change in the other groups. Naphthalene appears to have reduced all groups equally drastically from 50 % in group 4 to 72 % in group 3.

VIII. Use of baits in estimating the density of the wireworm population

An important paper by Miles & Petherbridge was published in 1927 describing experiments with baits such as wheat, oats, potatoes and bran, as a means of assembling wireworms, attracting them from the roots of crop plants upon which they were feeding. The assembled insects were then killed by the application of calcium cyanide.

Miles & Petherbridge showed that there was considerable variability in the degree of attractiveness of various baits, potatoes being least attractive and wheat the most attractive. The preliminary investigation of an area of 4 acres, a typical Lincolnshire silt, made by taking 83 soil samples 6 in. square to a depth of 9 in., gave a total of 81 wireworms, about 170,000 per acre, the majority located within the top 3 in. of soil. The baits were set in rows 10 yd. long and 5 ft. apart, at a depth of 2–4 in., 21 rows in all. After 14 days the whole row was taken up and sifted for wireworms. In the total area 972 wireworms were assembled, representing 13,000 per acre, or 7·7 %. Further experiments showed that:

(*a*) Baits at 3–4 in. deep were more efficient than those at 2 in. (*b*) Bran and wheat attracted wireworms most speedily, while oats, peas, beans and potatoes increase in attractiveness from 6 to 12 days after setting. (*c*) From 60 to 80 % of the wireworms present in any area can be attracted to the baits, depending on the type of soil and crop grown. Calcium cyanide, applied at the rate of 2–3 lb. per 100 yd. of the bait row, killed 75–100 % of the wireworms assembled.

It would be a great saving of time and trouble if the density of the infestation on a set of plants could be ascertained by baiting instead of by direct counts on the soil. This would be possible only if the number of wireworms caught by the baits was a definite proportion of the population.

Experiment 1

The first experiment was made with a smaller concentration of bait than Miles & Petherbridge and using potatoes which they had found to be the least attractive, but convenient to handle. The experiment was done on the Knott Wood plots in July 1935. There were 25 plots altogether, each of $\frac{1}{60}$ acre. Crop, sugar-beet. Half-potatoes were used as baits. The baits were placed in between the rows of sugar-beet, about 3 in. from the surface, each plot being divided into three sections. Four baits at positions determined by random numbers were placed in each of three rows in each section, making twelve baits per section and thirty-six baits per plot. After 10 days the baits were lifted and examined, any wireworms being removed and any bad potatoes replaced by fresh ones. After a further period of 10 days the baits were lifted and examined once more. The experiment employed two men for 48 hr., being much less than the time occupied by actual counts on soil samples.

A comparison of the number of wireworms obtained by baiting with direct counts on the soil samples showed however that there was no simple relation between them, thus:

No. of wireworms (total of 5 plots)

Treatment	Direct counts (30 soil samples, 9 × 9 in.)	In baits: Actual	In baits: Per 100 direct count
Nil	79	56	71
Chlorpicrin	67	57	85
Chlordinitrobenzene	109	39	36
"*K*"	42	32	76
"*M*"	64	38	59
Mean	72·2	44·4	61·5

Looking at the last column, it is seen that for 100 wireworms found in the soil samples the number caught in the baits was not constant, although the ratios on the "*K*" plots and untreated plots are very similar, 76 and 71.

The experimental error was 40·1 % compared with 49·8 % by direct sampling. "*K*" gave the lowest number of wireworms both by the direct count and by baiting, and in both cases the reduction in numbers compared with the controls was nearly significant.

Experiment 2

High Field II, 48 plots of $\frac{1}{200}$ acre.

Here there was a much higher population and no crop to compete with the baits for the wireworms. In consequence more wireworms were obtained, although the number of baits per unit area was the same as in the first experiment.

Potato and cabbage-leaf baits were used, six of each kind per plot in two lines of three, both the locality of the lines and the positions of the bait in the lines being determined by randomization beforehand. The baits were put down on 22–24 May and examined on 27–31 May. Much rain fell between 22 and 27 May, so that the baits from blocks 1 and 5 that were examined on the day were very wet, probably causing fewer wireworms to be attracted. The baits were replaced and examined again on 5–7 June. The time occupied was about 62 hr. for a total of 576 baits, 9·29 baits per man per hr. This agrees very well with the time taken by the first experiment.

Table XV shows the number of wireworms caught in the different baits and compares these numbers with the population found by direct

Table XV

Comparison of bait catches with direct counts of numbers of wireworms

Total of 8 plots

Treatment	Direct counts (16 soil samples, 6 × 6 in.)	Baits: Actual Pot.	Baits: Actual Cab.	Baits: Actual Total	Baits: Per 100 direct Pot.	Baits: Per 100 direct Cab.	Baits: Per 100 direct Total
Nil	126	52	133	185	41	105	146
Lime	117	42	98	140	36	84	120
Tar + lime	92	79	116	195	86	126	212
Ammonium carbonate	90	37	97	134	41	108	149
Superphosphate	113	43	66	109	38	58	96
Naphthalene	65	48	110	158	74	169	243
Mean	100·5	50·2	103·3	153·5	50	103	153

counts on the soil. On the average the cabbage-leaf proved twice as attractive as the potato. From the analysis of co-variance there does not appear to be any real relationship between the number of wireworms obtained by the baits and the actual number in the soil. However, the highest proportion of the actual number of wireworms was obtained in the case of naphthalene, and tar + lime both with potatoes and cabbages. This indicates a repellent effect sending the wireworms into a more congenial environment, and considerably reducing any hopes that this baiting method could be used as a substitute for direct sampling in fumigation experiments unless a very large number of baits were used.

The object was not to trap the wireworms and destroy them as in Miles & Petherbridge's experiments, but it is interesting to ascertain whether the baits have captured an appreciable proportion of the wireworms infesting the plots. The total population calculated from direct soil samples is 225 wireworms per sq. yd. or 1,089,000 per acre. Of these, a total of 921 were obtained in the baits (potatoes and cabbages) from 48 plots of $\frac{1}{200}$ acre, equivalent to 3838 per acre, or 0·35 % of those found to be present in the soil by direct count, whereas Miles & Petherbridge obtained 7·7 % in their first experiment and from 60 to 80 % in subsequent trials. If the area of the paths is included in our experiments the proportion is considerably less than 0·35 %. Thus the baits have had no appreciable effect on the total number of wireworms present in the soil.

SUMMARY

1. This paper describes an attempt to find out whether it is possible to test chemical control measures against wireworms by a field technique similar to that used in fertilizer and varietal experimentation. A full account is given of three field experiments using old grassland on a heavy "clay-with-flints" soil .

The arrangements and sizes of the plots were as follows: (1) 5×5 Latin square, $\frac{1}{60}$ acre; (2) 3×10 randomized blocks, $\frac{1}{70}$ acre; and (3) 6×8 randomized blocks, $\frac{1}{200}$ acre.

2. Wireworm populations were ascertained in every case by sampling the soil before and after treatment. The Ladell flotation machine was found rather too small and a modification of the technique using oil drums was adopted. Later, a larger and improved form of the machine was used.

In the first experiment six samples ($9 \times 9 \times 5$ in.) were taken in each plot, making a total of 150 soil samples on each occasion. In the second,

four samples ($9 \times 9 \times 5$ in.) per plot were taken, making a total of 120 before treatment, but after treatment the size of the samples was reduced to $6 \times 6 \times 5$ in., the number of samples remaining as before. In the third experiment two samples ($6 \times 6 \times 6$ in.) per plot were taken, totalling 96 on each occasion.

Local control was introduced in the first two experiments. In addition preliminary experiments were carried out using potatoes and cabbage-leaf as baits as a means of estimating wireworm populations. No relationship was found between the number of wireworms obtained in the baits and the actual number in the soil.

3. The wireworms were grouped according to size in order to ascertain if there was any differential action of the fumigants on the various instars. No conclusive evidence was obtained.

The mean density of the original wireworm population was 65, 335 and 277 per sq. yd. respectively in the three experiments.

The uneven distribution of the wireworms in the soil resulted in high sampling errors accounting for most of the experimental error. This aspect of the work is discussed fully by W. G. Cochran in the Appendix. No relation was found between the pH of the soil and the density of the wireworm population.

4. The untreated controls showed changes of -23, $+1$ and -9% between the first and second sampling respectively. The biggest drop was obtained when the second sampling was done in July and the smallest difference when the second sampling was done in May. It is suggested that the big drop in July might be due to a downward movement of the wireworms to escape the heat, but the evidence is insufficient to prove this point.

5. Fumigants "*K*" and "*S*" (mixture of *o*- and *p*-dichlorbenzene) reduced the population by 69 and 62 %, but had an adverse effect on the crop (sugar-beet) in both cases, due to insufficient interval between its application and the drilling of the seed. There was also a residual effect the following year, a reduction in the number of the wireworms and an increase in the plant stand (barley), although there was no significant increase in the final crop yield.

6. Chlorpicrin reduced the population by 46 and 33 %, but there was no harmful effect on the crops (sugar-beet and barley). No residual effect was detected.

7. Fumigant "*M*" (sodium cyanide) reduced the population by 45 % and increased the yield of the crop (sugar-beet). The residual effect was negligible.

8. Crude naphthalene ("creosote salts") reduced the population by 60 %, tar + lime by 29 % and ammonium carbonate by 20 %, on summer fallow.

9. Other treatments which all proved ineffective were chlordinitrobenzene, lime alone, and superphosphate.

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APPENDIX

THE INFORMATION SUPPLIED BY THE SAMPLING RESULTS

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1. *Introduction*

As the quotations at the beginning of Ladell's paper indicate, previous writers on the subject of wireworm control fully realized the need for estimating the wireworm population, but appeared to have no figures from which to assess the amount of work required to obtain a reasonably accurate estimate. An attempt to obtain such data was made by Jones (1937), who took samples with surface areas of 1, $\frac{1}{4}$ and $\frac{1}{16}$ sq. ft. respectively from a number of fields and compared the standard errors per sample. Jones finds, as one would expect, that the accuracy per sample increases as the size of the sample is increased; unfortunately, however, he does not balance this gain against the extra work required in taking larger samples, so as to find which size gives the best results per unit of work expended.

When a field experiment on the control of wireworms is under consideration, a preliminary sampling of the type which Ladell undertook is essential to determine the amount of work which is likely to be involved in estimating the effects of the treatments on the numbers of wireworms. The points on which a preliminary sampling may be expected to supply information are: (1) What size of treatment effect can we hope to detect with the amount of work done in the preliminary sampling? (2) If the treatment response which will be detected is considered too large, by how much must the sampling be increased to detect a treatment response of given size? If the standard of accuracy aimed at is found to involve too much labour, the postponement of the experiment must be seriously considered. (3) In a replicated field experiment of the type carried out by Ladell, the accuracy may be increased either by increasing the number of replications or by increasing the amount of sampling per plot. Which is the more profitable?

The purpose of this note is to show how the sampling technique used by Ladell enables us to answer these questions. We will consider in detail the results of the first sampling, which are given in Table I, p. 344.

When the experiment was started, it was of course unknown whether the distribution of wireworms was a random one over the whole experimental area. The experimental design used, a Latin square, was chosen

to take advantage of any regular gradients of infestation which might exist throughout the site, since differences in wireworm population between whole rows or columns do not affect the treatment comparisons. Further, the six soil samples taken per plot were restricted so that three fell in the north half and three in the south half of the plot; thus differences in infestation between these halves do not influence the treatment comparisons. This type of restriction, known as local control, is always worth while with new work, since one cannot lose anything in accuracy by it, and may gain substantially. The only limitation to its use is that at least four samples per plot are required to estimate the sampling error.

2. *The analysis of variance*

Before discussion, a complete analysis of variance is required. Owing to the small numbers of wireworms obtained per sample, their distribution is by no means normal, and before analysis the data ought to be transformed to some scale, such as square roots, on which they are approximately normally distributed. However, to keep the example as simple as possible, the analysis will be made on the numbers themselves; the conclusions are not altered thereby in this particular case.

The variation may be divided into: (I) Between-plot variation, which consists of variation between whole rows, with 4 degrees of freedom, variation between columns, with 4 degrees of freedom and the experimental error, with 16 degrees of freedom.* (II) The variation within plots between half-plots, derived from the differences of the totals of the north and south halves of a plot. As mentioned above, this variation does not enter into the experimental error, but it is worth calculating to see how much, if anything, has been gained by the local control. (III) The variation within half-plots, which constitutes the sampling error.

The first part, (I), is the ordinary analysis of variance of a Latin square and its calculation will not be given in detail here, as it is described with full numerical working in many text-books, such as that by Fisher & Wishart (1930). The ordinary "treatments" and "error" terms should be combined, as there are no treatments. If, however, the first sampling contained different treatments, the error term alone would be used. This analysis will be on a single plot basis (total of six samples).

To obtain (II), first take the differences (ignoring sign) between totals of the south and north halves of each plot. These are shown in Table I in the columns headed (S − N). The sum of the squares of these twenty-

* Plots 103–107 form the first row, and plots 103, 108, 113, 118 and 123 form the first column, etc.

five differences is 2269 and is on a single plot basis. The sampling error (III) may be obtained by a subtraction. Calculate the sum of the squares of all 150 samples; this comes to 3767 and is on a single *sample* basis. Multiplying by 6, to bring this to a single *plot* basis, gives 22,602. Subtract the product of the grand total, 607, and the general mean 24·28. This gives the total sum of squares 7864·04 with 149 degrees of freedom. The sampling variation may now be obtained by subtracting (I) and (II) from the total and has 100 degrees of freedom.

The complete analysis of variance is as follows:

	D.F.	Sums of squares	Mean square	S.E.
Rows	4	515·44	128·86	—
Columns	4	523·44	130·86	—
Experimental error	16	712·16	44·51	6·672
Between half-plots	25	2269·00	90·76	—
Sampling error	100	3844·00	38·44	6·120
Total	149	7864·04		

3. *The information supplied by the preliminary sampling*

The first point to notice is that the experimental design considerably improved the accuracy of the results, since the mean squares due to rows, columns and differences between half-plots are all substantially above the experimental and sampling mean squares. Had the experiment been randomized completely within the site chosen, on the ground that the wireworm distribution was a random one, the experimental error (with local control) would have been

$$\tfrac{1}{24}(515{\cdot}44+523{\cdot}44+712{\cdot}16)=72{\cdot}96 \text{ instead of } 44{\cdot}51.$$

Further, if no local control had been used, the sampling error would have been

$$\tfrac{1}{125}(2269+3844)=48{\cdot}90 \text{ instead of } 38{\cdot}44.$$

Thus an estimate of the experimental error with complete randomization and no local control is

$$72{\cdot}96+49{\cdot}90-38{\cdot}44=83{\cdot}42 \text{ instead of } 44{\cdot}51,$$

so that the accuracy of the experiment has been nearly doubled by the design.

The experimental error is 6·672 per plot and the standard error of a treatment mean (5 replicates) is $6{\cdot}672/\sqrt{5}=2{\cdot}98$, which is 12·3 % of the general mean (24·28). Thus the standard error of the difference between two treatments is 17·4 % of the general mean. To find the percentage difference which would be detected at the 5 % level of significance, this must be multiplied by 2·120, the value of "t" for 16 degrees of freedom.

Thus an apparent difference of 37 % between two treatment means will be significant.

When we are aiming at a given standard of accuracy, a further point, slightly more subtle, must be appreciated. If the *true* difference between say one fumigant and the control were 37 %, then in a number of experiments the estimated difference would vary about this value, being above it in half the experiments and below it in half. Thus in an individual experiment, a *true* difference of 37 % has only a chance of one in two of being detected as significant. The question arises, how large must the true difference be so that it will almost certainly be detected, say in nineteen experiments out of twenty? The difference must clearly be so large that only in 5 % of cases will the observed value of t fall below 2·120.

If x is the observed treatment difference, s its estimated standard error and μ the real treatment difference, then the tabulated $t=(x-\mu)/s$ and we want the value of t which is exceeded in all but 5 % of cases. From the t-table for 16 degrees of freedom we see that the value of t lies inside the limits $\pm 1{\cdot}746$ in all but 10 % of cases. Since the t-distribution is symmetrical, the value must therefore exceed $-1{\cdot}746$ in all but 5 % of cases. Thus the real difference must be such that when $(x-\mu)/s=-1{\cdot}746$, then x/s, the observed t, is 2·120. This gives $\mu=3{\cdot}866\,s=16{\cdot}29$, which is 67 % of the general mean.

Thus if we wish to be reasonably certain of detecting a response to a treatment, the response must be at least 67 %. This answers the first question in the introduction.

To consider the second question, suppose that we wished to detect a difference of 50 % in 95 % of cases. The standard error per plot would have to be reduced to $\frac{50}{67}$ of its present value and the experimental variance reduced in the ratio $(\frac{50}{67})^2$, i.e. to 24·8.

The third question concerns the best way to do this. The sampling variance, 38·44, accounts for all but 6·07 of the experimental variance. Thus doubling the sampling per plot, but keeping the size of the experiment fixed, would reduce the experimental error by $\frac{1}{2}$ (38·44) = 19·22, i.e. to 25·3. On the other hand, keeping the *total* amount of sampling fixed, but doubling the size of the experiment, would reduce the experimental error by only $\frac{1}{2}$ (6·07) = 3·04, i.e. to 41·47. There is thus a much smaller return from doubling the experimental area (using say two 5×5 Latin squares) than from doubling the amount of sampling. This must, however, be balanced by the experimenter against the expenditure of time and labour in doubling the sampling and the experimental work. It is

probable that with this type of work the former will be the more exacting operation and may even be a limiting factor to the size of the experiment.

In any investigation in which the major portion of the work is taken up in handling samples, the best return for the labour expended is obtained when the sampling variance accounts for all, or almost all, the experimental variance.

The only change in Ladell's technique suggested as a result of the preliminary sampling was to increase the local control at the next sampling by taking two samples per third of a plot instead of three per half-plot. The precision attained on the treatment comparisons was considered sufficient, as only large treatment effects would be of commercial interest, and in any case the experiment was regarded as one mainly on technique. As the experimental error was almost entirely due to variation between samples, no reduction in the amount of sampling could be recommended.

4. *The experimental and sampling errors*

The results of Ladell's experiments from the point of view of sampling technique are summarized in Table II. In most cases a high proportion of the experimental error was due to errors in sampling. As the amount of sampling was the limiting factor in these experiments, this result is gratifying and means that the labour of sampling could not have been decreased without a considerable sacrifice in accuracy. The experimental errors are high; nevertheless certain significant treatment effects were detected.

The amount of sampling varied from $\frac{1}{7}$ to $\frac{1}{2}$ % and the areas within which the sampling variation was taken ranged from $\frac{1}{200}$ to $\frac{1}{120}$ acre.

For comparison with other workers' data the sampling errors are best expressed in terms of a single sample, as shown in the table below.

Sampling errors per sample

Size of sample in.	Size of area sampled acres	W Number of wire-worms per sample	E Sampling error % per sample	$E\sqrt{W}$
9×9	$\frac{1}{120}$	4·0	61	122
9×9	$\frac{1}{180}$	2·4	83	129
6×6	$\frac{1}{120}$	1·2	96	105
9×9	$\frac{1}{140}$	21·0	38	174
6×6	$\frac{1}{140}$	6·2	48	119
6×6	$\frac{1}{200}$	7·7	74	203
6×6	$\frac{1}{200}$	6·3	72	181

When an experiment of this type is contemplated with new material of unknown variability, it is sometimes useful to note that a lower limit

to the sampling error may be obtained from theoretical considerations alone. If the wireworms were scattered at random throughout the sub-plots sampled, the numbers found per sample should be distributed in a Poisson series distribution. For this distribution the mean W is equal to the variance, so that the standard error E % per sample should be $100/\sqrt{W}$. Other sources of variation may, of course, increase this error.

The values of $E\sqrt{W}$ for Ladell's experiments are given in the last column of the table. They show quite a close agreement with theory in the first experiment, where the numbers per sample were very small, but with higher numbers per sample other sources of variation become important.

Jones's figures agree remarkably well with these, despite the fact that he was sampling from much larger areas. For the 25 sets of 100 sq. ft. samples in his Table III, the means ranged from 0·22 to 10·78 per sample and the values of $E\sqrt{W}$ from 85 to 237. The values of $E\sqrt{W}$ also increase as the means increase; in fact, apart from one or two widely aberrant sets, a good summary of Jones's results may be obtained by taking a linear regression of the form

$$E\sqrt{W}=a+b\sqrt{W},$$

where a and b are constants.

This agreement between results obtained under widely differing conditions gives one confidence in recommending Jones's and Ladell's sampling errors to workers who are planning field experiments on wire-worms and wish to obtain a preliminary idea of the scale on which they will have to sample. This scale should, of course, be reconsidered as soon as the results of their first sampling are available, by the method indicated in section 2. It should be noted, however, that Jones's sampling technique, which involved a systematic rather than a random distribution of samples, is not recommended for sampling work in field experiments.

Summary

In any field experiment which involves sampling of a laborious nature, it is important to know as soon as possible what degree of accuracy in the treatment mean values will be reached with a given amount of work, how much work must be done to reach a given standard of accuracy and how best to distribute one's resources between the amount of sampling and the amount of replication.

The first sampling, whether it contains experimental treatments or is uniformly treated, can supply information on all these points if

properly carried out. Ladell's first wireworm sampling is taken as a simple numerical example of the way in which these questions can be answered with the help of an analysis of variance.

The sampling and experimental errors of Ladell's experiments are discussed. The sampling error accounts for a large proportion of the experimental error in most cases, as it is always advisable where the labour involved in sampling is high.

Ladell's sampling errors agree well with those obtained under widely different conditions by Jones, and both may be recommended to other workers as an indication of the amount of variability to be expected in field sampling for wireworms.

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INVESTIGATIONS INTO THE NUTRITION OF THE ASH-BARK BEETLE, *HYLESINUS FRAXINI* PANZ.

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Introduction

No work has been done so far on the physiology of the nutrition of the bark-feeding Coleoptera. This is not surprising, considering that it is only very recently that attention has been given to the nutrition of some of the wood-feeding insects proper, which were described by Uvarov (1928) as "insects of entirely unknown feeding habit". Since then, papers by various workers have dealt with investigations into the nutrition of some of the wood-borers, but the nutrition of the bark beetles (Scolytidae) has never been studied despite its great interest, and the suggestion by Escherich (1923) and Munro (1926) that bark-beetle attack is only a secondary symptom confined to trees already in a pathological condition and is very rarely found in sound trees. It is a well-known fact, used in the control of the pest, that the insects go to felled trees if available rather than to living ones (Escherich). If it were possible to get a clear knowledge of the substances serving as their food, of the attraction of the insect by the plant, and the physiological relationship between them, new aspects of the ecology of both plant and insect would be opened, which might lead to better methods of control.

Accounts of the life history and anatomy of *Hylesinus* (*Leperisinus*) *fraxini* can be found in the writings of Escherich (1923), Munro (1926) and others. There are two distinctly different types of nutrition:

(1) "Brutfrass" which is the nutrition of the larva, and with which is linked up the "Ernährungsfrass" of the adult.

(2) "Reifungsfrass" which serves the young imago to reach sexual maturity.

Only the first type of nutrition is dealt with in this paper. The carbohydrate metabolism by frass analysis was studied quantitatively only in the larvae. However, the similarity of the enzymes and the

qualitative results of the analysis lead to the conclusion that there is no fundamental difference between larval and adult metabolism.

The nutrition of the ash-bark beetle, *Hylesinus fraxini*, has been studied in two ways:

(1) By an investigation into the enzymes of the alimentary canal.

(2) By a comparative analysis of the bark and of the frass of the insects, the word "frass" here meaning the total contents of the food tunnels.

Sources of error

These may be considerable in this kind of work, and have to be taken into account when assessing the value of the results. The following have to be considered:

Inaccuracy of methods. The biochemical methods employed are not very well worked out, and are usually very specific; the presence of other substances may affect results to a large extent, and the variation between duplicates or triplicates of the same estimation is usually considerable.

Variation in material. Though care was taken to eliminate this factor as much as possible by taking frass and bark always from the same pieces, and by making as many estimations as possible, it must be remembered that the chemical constitution of plants varies according to age, position, etc., to an unknown degree.

Metabolism of the material. As seasoning of the wood proceeds chemical changes occur in it, such as the breakdown of certain reserve carbohydrates. It is often difficult to decide how much of this breakdown is due to the metabolism of the bark and how much to the digestion by the insect.

Bacterial action. The frass in its partly broken-down form is much more susceptible to bacterial attack than the original bark. It is impossible to say to what amount changes have occurred in it after it has left the insect gut.

Composition of food-tunnels. These in places pass into the sapwood. Although there is probably not much sapwood passing through the gut, all results may be somewhat too high in their difference from the original bark, as the cellulose content, which has been made the basis of the calculation (see later), is higher in sapwood than in bark. No material was taken from the distal end of the food-tunnels, where the larva bores right into the sapwood to pupate.

Investigation of the enzymes of the alimentary canal

For these experiments a tissue suspension was prepared in the following way. For each test the guts of about twenty larvae or adults were dissected out and ground up with a few drops of distilled water in a solid watch-glass by means of a glass rod to give about 0·5 c.c. The suspension had $p\mathrm{H} = 7{\cdot}6$, which was found to remain unchanged in all tests after addition of the substrate without buffering, except in the peptase and hemicellulase tests. The suspension was divided into equal parts in two small glass tubes, one of which was heated for a few minutes in a boiling-water bath to kill the enzyme, for control. Equal amounts of the substrate were then added to each of the tubes together with a drop of toluene to prevent bacterial action, and both tubes were left in an incubator at 37° C. for 48 hr., after which time they were tested.

The following tests were carried out:

Peptase.

(*a*) A few particles of carmin-fibrin were added to the tissue suspension. If digestion took place the carmin would be liberated into the solution. The solution was kept acid with 2 drops of $N/10$ acetic acid (Cole, 1933, Ex. 262).

(*b*) An equal amount of acid casein solution, prepared according to Cole (1933, Ex. 261), was added to the suspension, and the test and its control were titrated with 10 % Na-acetate until a precipitate was obtained. If digestion took place a precipitate would be obtained later in the test than in the control, as casein would have disappeared from the former.

Tryptase.

(*a*) A few particles of Congo-red fibrin was added. Coloration test as under Peptase (*a*) (Plimmer, 1915, p. 474).

(*b*) Casein test: a neutral casein solution was added in equal parts to the suspension. Precipitation test with 1 % acetic acid (Cole, 1933, Ex. 273).

Peptidase.

Equal amounts of 1 % peptone solution were used as substrate. The disappearance of peptone from the digest was tested with the Biuret test (Cole, 1933, Ex. 277).

Lipase.

An emulsion of olive oil in water was used as substrate. The appearance of fatty acids through the action of lipase was tested by decoloration

of phenolphthalein and, if necessary, titration with $N/50$ NaOH (Plimmer, p. 475; Cole, Ex. 184).

Saccharase.

1 % cane-sugar solution was used as substrate. Tested by reduction of Benedict's solution.

Maltase.

1 % maltose solution was used as substrate. The osazone test (Plimmer, p. 265; Cole, Exp. 139) was carried out with the digest. If cane sugar was broken down, crystals of glucosazone would appear in the test.

Lactase.

1 % lactose solution was used as substrate. Osazone test as above.

Dextrinase.

1 % dextrin solution was used as substrate. Osazone test as above.

Amylase.

1 % starch solution was used as substrate. The disappearance of starch was tested with iodine solution.

Amylo-hemicellulase.

This test was carried out to test the behaviour of the gastric juices to α-glucans of higher molecular weight than starch. A few particles of amylo-hemicellulose (see Clayson & Schryver, 1923) were added to the suspension, and digestion was tested with iodine solution as in the case of starch, the substrate giving a bright blue coloration in this test.

Hemicellulase.

Hemicellulose "B" from ash bark, prepared in the course of the chemical analysis, was used as substrate. The suspension was buffered to $p\mathrm{H} = 7{\cdot}6$ with acetate buffer. Digestion was tested for with Tollens's test and Benedict's test.

Cellulase.

(*a*) A suspension of filter-paper was used as substrate and the test made with chlor-zinc-iodine solution (Schulz solution).

(*b*) Sections through the stem of a green plant were exposed to the actions of the enzymes of the suspension, and later stained with Schulz solution, and examined under the microscope. Failure of the cell walls to stain purple indicated that the cellulose in them had broken down.

(*c*) A few milligrams of finely divided cellulose pulp ("poudre" Prat, Courze) were weighed into a known quantity of suspension and, after

digestion, any reducing sugars present were estimated by reduction following Bertrand's method.

The results of all these tests are expressed in Table I.

Table I

Determination of enzymes of Hylesinus fraxini

Enzyme	Result	Enzyme	Result
Peptase	Absent	Lactase	Very weak
Tryptase	Present	Dextrinase	Present
Peptidase	Present	Amylase	Weak
Lipase	Weak	Amylo-hemicellulase	Very weak
Saccharase	Present	Hemicellulase	Present
Maltase	Present	Cellulase	Absent

After the chemical analysis of the bark had shown that starch had disappeared from the bark before it is attacked by *H. fraxini*, it seemed advisable to measure the quantitative strength of the group of enzymes attacking α-glucans, to investigate whether there was a connexion between the delay in attack (i.e. until the disappearance of the starch) and the strength of the enzymes. At the same time it was thought desirable to compare the strength of the hemicellulase with that of the lactase, as galactan forms the greater part of the hemicellulose. In view of the fact that some authors, such as Bernhauer (1933), show galactan with the β-linkage, it was thought possible that the enzyme in question would be most nearly related to that attacking lactose, i.e. a β-galactoside. For these experiments the guts were treated as before, and an equal amount of the suspension measured into each tube with a micropipette. The substrate was weighed in. After 48 hr., all reducing substances were measured by the Bertrand method. The difference in reduction between test and control gave the action of the enzyme.

The result is expressed in Table II.

Table II

Quantitative action of several carbohydrases

Material	Weighed in g.	Reduction after 48 hr. in glucose g.	Net reduction due to enzyme %
Dextrin	0·032	0·030	90
Control	0·035	0·014	—
Starch	0·032	0·018	56
Control	0·033	0·000	—
Lactose	0·029	0·019	27
Control	0·033	0·017	—
Hemicellulose "B"	0·030	0·009	30
Control	0·038	0·000	—

Comparative analysis of bark and frass

A. *Carbohydrates*

The utilization of carbohydrates by wood-feeding insects has been studied by the method of comparative analysis by Campbell (1929) working on *Lyctus* spp. and *Xestobium rufovillosum*, and by Norman (1936) working on *Xestobium*. Part analysis has been carried out by Ripper (1930) working on some cerambycid larvae and *Xestobium*, by Falck (1930*a*, *b*) in work on the cellulose digestion of *Hypotrupes bajulus* and of *Anobium*, and by Mansour & Mansour-Bek (1934*a*, *b*) in their study of the nutrition of *Macrotoma palmata* and *Xystrocera globosa*.

For the present investigation, ash bark was ground up to pass a 60-mesh sieve but to be retained by a 90-mesh sieve. The frass of the larva passes 60 mesh easily, but is partly retained by 90 mesh. The greater part of the adult frass is retained by a 60-mesh sieve. General appearance of the particles and the general analysis suggests that only part of the adult frass has passed through the gut. It is not definitely known how much of the larval frass actually passes through the gut, but it may be assumed that the greater part does so.

The methods employed are given in the text-book by Hawley & Wise (1926) on the chemistry of wood, and the structure and properties of the carbohydrates concerned and their biochemical metabolism has been described by Bernhauer (1933) and others. An account of the chemistry of the major carbohydrate constituents of ash bark and the method for their estimation has already been given elsewhere (Buston & Hopf, 1938) and only the general methods are here briefly outlined.

The following fractions of the material were distinguished:

(1) Hot-water-soluble material, containing all the simple sugars, together with dextrins, glucosides and wood starch (cf. Campbell, 1935).

(2) Pectic substances, obtained by extraction with 0·5 % ammonium oxalate.

(3) Alkali-soluble substances, mainly hemicellulose.

(4) Cellulose (including inseparable pentosans).

(5) Lignin.

The material was first extracted with water under a reflux condenser at about 90° C. four to six times for 5 hr. each. The extraction with ammonium oxalate was made at the same temperature for 6 hr. The remaining substance was extracted with $N/1$ NaOH several times (until the extract was colourless), 5 hr. each.

The water-soluble group was further subdivided by adding nine parts of alcohol to one part of the extract, which throws down all the higher polysaccharides. After filtering off, reduction estimations were made with the filtrate before and after hydrolysis with weak sulphuric acid, giving the amounts of simple sugars (monosaccharides) and of the more complicated (disaccharides, simple oligoses) respectively.

The alkali-soluble fraction was first acidified to litmus with acetic acid, the precipitate of hemicellulose "A" thus obtained filtered off, and five parts of alcohol were added to the filtrate. A precipitate of hemicellulose "B" was obtained. This was filtered off, and the same amount of alcohol was added again, giving a precipitate of hemicellulose "C". The separation of "B" and "C" can naturally not be regarded as quantitative. In each hemicellulose the amount of uronic anhydride was measured by determining the carboxyl groups, and the pentosan constituents by the furfural method of Tollens, as modified by Norris & Resch (1935). (See also Preece, 1931; Angell *et al.* 1936.) The furfural due to urone was allowed for in this estimation. The rest was regarded as consisting of hexosans. Lignin was estimated by Willstätter's method; cellulose by chlorination according to Cross and Bevan's method.

The same methods of fractionation and analysis were applied to the frass and the bark.

To determine the actual loss in weight of the frass by passing through the insect gut, it was assumed that cellulose, which was found to be unattackable by the gastric enzymes, remained constant. As the cellulose-lignin ratio seemed to remain constant within the limits of error this assumption seemed justified.

There was some variation in the different specimens of bark investigated, according to the age of the attacked specimen, its moisture content while exposed after felling, and the progress of the seasoning metabolism. The summary of a typical example, which represents a fair average of the determinations made, is given in Table III.

The simple sugar fraction seems to consist mainly of glucose. It is difficult to say anything definite about the nature of the alcohol-soluble, and even less about the alcohol-insoluble, polysaccharides. However, two facts seem to be important in this connexion. First, the bark in the state in which it is ready for insect attack contains no starch. The iodine test was always negative. Fresh ash bark always shows a clear reaction. The disappearance of starch in felled timber by seasoning has been recorded before by Mer (1903), Wilson (1930) and Henderson (1935). Second, the alcohol-insoluble polysaccharides of the unattacked bark in fresh trees

contain little or no pentose constituents. With the seasoning of the wood, pentose appears in increasing quantities in this fraction, probably derived from the breakdown of reserve material, chiefly hemicelluloses. However, the frass always contains more pentose than the bark and shows a positive reaction to the orcinol test at a time when this fraction from the unattacked bark seems to be still practically free from pentose. It is most likely that the fraction consists mostly of α-glucans of the dextrin type, though this could not be proved either by acid-hydrolysis or by the takadiastase method described by Denny (1934) which Campbell (1935) employed successfully.

Table III

Analysis of bark and frass (general)

	Material		
	Bark %	Frass %	Frass in % of original bark* %
Water-soluble fraction:			
Simple sugars	5·32	4·85	4·00
Alcohol-soluble polysaccharides	4·86	1·65	1·36
Alcohol-insoluble polysaccharides	8·05	6·2	5·12
Other substances	11·52	13·6	11·2
Total	29·75	26·3	21·7
Alkali (4% NaOH)-soluble fraction:			
Hemicellulose "A"	2·65	3·55	2·93
Hemicellulose "B"	13·6	10·65	8·78
Hemicellulose "C"	3·12	1·8	1·49
Total hemicellulose	19·37	16·0	13·2
Pectin (as Ca-pectate)	3·24	4·6	>3·8
Lignin	16·5	21·5	17·5
Cellulose	32·5	39·4	32·5
Total ash	6·42	7·3	6·01
Total pentosans	10·6	12·2	10·08
Protein†	2·88	1·43	1·18

* Based on the assumption that cellulose remains constant, in which case frass = 82·5% of original bark.
† See Table V.

The group comprised in Table III under the heading "other substances" in the water-soluble fraction probably consists of material other than carbohydrates together with those carbohydrates which have resisted 12 hr. hydrolysis. No details whatsoever (except for the proteins) about its nature are available.

The pectic substances consist for the greater part (over 80%) of pure polyuronides. The values given are calculated as calcium pectate and are therefore not true percentages of the material.

The hemicelluloses of the ash bark are built up of three main constituents: galactan and mannan (hexosan), araban, and uronic acid. They differ mostly according to the distribution of these groups and by their molecular size.

The utilization of the hemicellulose constituents will be seen from the figures in Table IV, where the percentages found in bark and in frass are given. The percentages of the unattacked bark have again been calculated under the assumption that:

$$\frac{\text{frass}}{\text{bark}}=\frac{\text{cellulose of bark}}{\text{cellulose of frass}}=\frac{32{\cdot}5}{39{\cdot}4}.$$

Table IV

Analysis of bark and frass (Hemicelluloses)

		Bark		Frass	
Hemi-cellulose	Constituent	% of respective hemi-cellulose	% of unattacked bark	% of respective hemi-cellulose	% of unattacked bark
"A"	Hexosan	—	—	—	—
	Uronic anhydride	—	—	—	—
	Pentosan	95	2·6	—	—
	Total	100	2·65	100	2·93
"B"	Hexosan	65	8·77	40	3·5
	Uronic anhydride	17	2·37	29·9	2·63
	Pentosan	18	2·46	30·1	2·64
	Total	100	13·6	100	8·78
"C"	Hexosan	65	2·03	—	—
	Uronic anhydride	20	0·62	—	—
	Pentosan	15	0·47	—	—
	Total	100	3·12	100	1·49

From the values giving the constituents in percentages of the dry weight of unattacked bark it will be seen that as the variations in the amounts of urones and pentosans are within the limits of error necessarily connected with work of that kind, the decrease in hexosans is very considerable and must be regarded as due to utilization of hexosan by *Hylesinus fraxini.*

The frass numbers have only been worked out for "B", as this is present in larger quantities and must necessarily play a greater role in nutrition than "C". However, the results should be the same in principle in both cases.

The cellulose was regarded as one unit and no distinction was made between the β-glucan proper and the pentosan groups linked up with them. From what is known about them and the results of the enzyme tests and about the pentoses of the alkali-soluble group, it seems very

unlikely that these molecular chains are attackable by the insects in question and that therefore further division is of no interest in this investigation.

The "total pentosan" value was inserted following Campbell (1929). It might better be described as the total yield of furfuralphloroglucide, were it all due to pentosans. In fact, some of it will be due to uronic anhydride, the amount of which it is impossible to determine because of the interference of organic acids etc.

B. *Proteins*

The results of this investigation have already been referred to in a letter to *Nature* (Hopf, 1937) dealing with the protein digestion of wood-boring insects.

The total nitrogen was first determined by the micro-Kjeldahl method. The material was then extracted for several hours with hot water to which some phenol was added and trichloracetic acid to throw down the protein. From the filtrate the non-protein was determined by the micro-Kjeldahl method, the difference being regarded as protein nitrogen. The protein number was obtained by multiplying this value by 6·25.

Table V

Analysis of bark and frass (*Proteins*)

Sample	Total N %	Non-protein N %	Protein N %	Protein %	% of unattacked bark
Ash bark	0·54	0·08	0·46	2·88	2·88
Frass of larva	0·41	0·182	0·288	1·43	1·18
Frass of adult	0·68	0·326	0·354	2·21	—

A considerable amount of ammonia was found in the excretions, which may have been derived from uric acid by bacterial action. In no case was there enough uric acid to account for the total excretory nitrogen. Urea was never present.

No work has been done on fat and similar substances in the bark, but they do occur there.

DISCUSSION

The sources of error mentioned above may certainly affect the quantitative details of the results, but it is hardly probable that they make a principal difference, especially as the two methods of investigation, i.e. the enzymological and the analytical, yield results pointing in the same direction.

Apparently *Hylesinus fraxini* is able to make use of a very varied supply of carbohydrates. There is a weak α-glucanase which, though it is strong enough to break down starch, is by no means as strong as that found in many other insects, where the iodine test in the digest is often negative after a few minutes. It is also strong enough to digest quantitatively and in a short time the carbohydrates similar to starch but of shorter chain-length, which are probably present in the bark in the form of dextrins and other oligoses. A considerable decrease is found in this fraction of the frass. The actual utilization of this group is certainly much greater than is indicated in the decrease in the alcohol-insoluble sugars, but this fraction is filled up again with breakdown products of the higher groups, i.e. the hemicelluloses, as the increased presence of pentoses in it in the frass indicates. As there is no starch present in the bark attacked, the trees in this state seem to be already in a pathological condition, which gives support to Munro's suggestion that the insect only attacks such trees as a secondary pest. Fresh ash bark always shows a positive reaction to the iodine test. It might appear, therefore, that the beetles are attracted to trees offering them, in the form of already partly broken-down starch, a food more easily digestible. This is underlined by the observation that α-glucosides of chain-length even greater than starch (amylohemicellulose) are still less attacked by the enzymes. The question remains open whether the starch breakdown as such, through fermentation or otherwise, supplies the attractant to the beetle.

It is interesting to contrast this behaviour with what is known about the feeding habits of some of the wood-boring insects proper. *Lyctus*, for instance, seems to feed mostly on starch. The plant-physiological work by Mer (1903), Wilson (1930), and Henderson (1935), the analysis by Campbell (1929), and especially the feeding and extraction experiments by Parkin (1936), have proved that *Lyctus* depends on an ample supply of starch and will not attack wood which is free from it.[1] Many other wood-boring insects also seem to depend on starch (*vide* Mansour & Mansour-Bek, 1934). In the case of the ash-bark beetle, the mode of nutrition is entirely different and it shows a decided preference for already seasoned wood, and will even attack an old specimen twice, chewing frass of the previous broods again, rather than attack fresh bark nearby.

[1] In this connection, it is interesting to draw attention to the statement by Munro (1928) that with the exception of *Hylotrupes bajulus* all the longicorn beetles commonly introduced into this country prefer unseasoned timber as a breeding ground, and for that reason they rarely increase in the timber yards, and the injuries they cause do not extend to other timber in the vicinity. *Hylotrupes* has been proved by Falck (1930*b*, see later) to utilize cellulose.

That there is a considerable loss of alcohol-soluble sugars of the disaccharide and similar types in the frass is quite natural. There are probably very powerful enzymes for these groups present, which split up all breakdown products from higher groups. The simple sugars do not decrease in the same proportion, showing that the enzymes split up more than the body is able to assimilate and that, therefore, a part of the digest is allowed to leave the gut with the frass. There is, of course, always the possibility that the presence of reducing substances other than sugars increases the values in this fraction, especially in the frass.

The utilization of the hemicelluloses is especially interesting as, according to a widely accepted theory, these bodies have arisen by a biochemical oxidation, which first transformed the alcohol groups of some links of a hexose chain into carboxyl groups yielding uronides, and then by further oxidation split off carbon dioxide to give a pentose link. As can be seen from Table IV, only the hexose (galactose) links are utilized. Their disappearance partly breaks the chain, so that pentoses appear together with carbohydrates of as small molecular weight as the water-soluble, alcohol-insoluble saccharides. The apparent increase in hemicellulose "A" can probably be explained by "B" and "C" break down products, containing much pentose and little urone, having thus acquired the approximate solubility of the "A" group which is less than that of "B" and "C."

Hemicellulases are well known in insects. Campbell (1929) and Norman (1936) found that *Xestobium* utilizes hemicellulose, but the only case where a hemicellulase has been more clearly defined in a wood-boring insect is that of the lichenase which Ullmann (1932) found in *Cossus cossus*—as lichenin may be regarded as related to the hemi-celluloses.

The process in the *Hylesinus* gut is probably this, that the enzymes split off the hexose units of the chain. The rest of this chain goes either into fractions small enough to appear in the water-soluble group, or in the hemicelluloses of smaller molecular weight, possibly in "A", or stays in "B" with a decrease of the hexose constituent. It seems quite feasible that part-oxidation during the process converts the urone constituents of the chain partly into pentose units, with corresponding decrease of solubility. Hence the increase in hemicellulose "A". "C" is relatively more strongly attacked than "B" as it is of smaller molecular size, but, as it is present only in small quantities, it does not play the same role as "B" in the nutrition.

The presence of only a weak lactase can be reconciled quite well with the utilization of the hemicelluloses. The two enzymes are probably quite different, especially as it is only assumed that we have to deal with

a β-galactanase. The breakdown may go directly to free galactose without passing through the units which necessitate a β-galactosidase.

Cellulose and carbohydrates of similar type are certainly not attacked. Enzymes attacking these complexes have never been recorded in insects of this type. Cellulose digestion is common in wood-boring insects and has been recorded in certain cerambycids (Ripper, 1930; Falck, 1930*b*; Mansour & Mansour-Bek, 1934*a*, *b*; Horn, 1932) and anobiids (Ripper, 1930; Falck, 1930*a*; Campbell, 1929; and Norman, 1936) by means of chemical analysis. It is not quite certain whether the digestion is due to enzymes secreted by the insects or by bacteria or other micro-organisms with which they live symbiotically. Cellulases seem to be present in certain cerambycids, but their presence in some other cases, in which they have been recorded, cannot yet be regarded as actually proved. I was not able to demonstrate the presence of the enzyme in locusts (Biedermann, 1919) or in the larva of *Anobium*, where Falck (1930*a*) records a disappearance of cellulose in the frass by analysis, while the method gave very clear results in the case of *Helix pomata* (Karrer, 1925).[1]

The utilization of the proteins from the bark seems to be in accordance with the strength of the enzymes and the manner in which the carbohydrates are attacked. It is a surprising fact that the adult beetle actually excretes more nitrogen than it takes up, but it must be remembered that the adult frass examined was obtained from beetles within 2–3 weeks after emergence from the pupa. It seems that the insect gets rid of the waste products of metamorphosis in a way resembling the deposition of the meconium in Lepidoptera. Possibly analysis of the "Reifungsfrass" of the young adults before mating and oviposition, would give quite different results, but as it was not possible to breed *Hylesinus* in captivity, insufficient material for this investigation was available. The whole question of the nitrogen supply of insects is still in need of further research. A definite discrimination between the different proteins available is required, and the problem of excretion, in particular, seems to offer a wide scope for further research. No final conclusions can be reached before this question is cleared up.

It is also necessary in the study of the ecological aspect of the question to establish definitely the connexion and interaction of the physiology of the plant and of the insect with regard to the nutrition of each. There may naturally be many other factors acting as attractants outside the starch breakdown, or even outside the food supply. An accurate and

[1] Since writing this paper Schlottke (1937) states that no cellulase occurs in *Saltatoria* investigated by him.

detailed investigation into the physiology and biochemistry of the plant both prior to and after attack would seem essential in order to get a satisfactory solution of the ecological problems. Nutrition in regard to life history ought also to be studied. For instance, it would be interesting to know why the larva bores into the sapwood before pupating, and whether there is a constituent in this part essential for the mechanism of metamorphosis. The knowledge of wood-chemistry on the one side and of sense-physiology on the other must be more advanced before the question of attraction can be seriously gone into. Feeding experiments with artificial diet might also yield some more information of the principles underlying bark-beetle nutrition.

Summary

From investigations into the enzymes of the alimentary canal and a comparative analysis of ash bark and the frass of *Hylesinus fraxini* it appears that the food relations of the ash-bark beetle are as follows:

(1) Proteins are derived directly from the bark. The adult after emergence excretes more nitrogen than it consumes; this excess excretion may be regarded as representing waste-products of metamorphosis.

(2) The carbohydrates are derived from the following bark constituents:

(*a*) The simple sugars.

(*b*) Disaccharides, simple oligoses etc., probably all with α-linkages.

(*c*) The α-glucans up to starch, but with a definite preference for sugars of smaller chain-length. Starch as such has disappeared from the bark of attacked trees.

(*d*) The hexosan parts of the hemicelluloses.

(3) The protein-hydrolysing enzymes are of the tryptase and peptidase type, in accordance with the alkaline pH of the gut.

(4) The carbohydrate-hydrolysing enzymes are of the following types: saccharase, a weak lactase, maltase, amylase (dextrinase), galactanase.

(5) Cellulose is clearly not attacked. The same applies to all pentosans.

(6) The breakdown of starch in the attacked bark may serve as an attractant to the bark beetles, and may help to explain their preference for trees suffering from physiological or pathological disturbance.

(7) A way has been shown in which the hemicelluloses are utilized by the insect in question, and the probable fate of the breakdown products of these substances in the frass has been indicated.

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THE STEM AND BULB EELWORM, *ANGUILLULINA DIPSACI* (KUHN), IN STRAWBERRY IN BRITAIN

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(With Plate XVII)

Introduction

Anguillulina dipsaci has a known host range of some three hundred different plants but, owing to the presence within the species of numerous biologic strains or races, the host range of any particular strain is often strictly limited (Hodson, 1931). Widespread as the nematode is in Britain there is, apparently, no previous authentic record of its attacking strawberry here, and the main purpose of this paper is to record such occurrence. As the strawberry is already a well-known host plant in the United States, a brief discussion of the American literature is desirable.

Historical

An early account is that by McKay (1921) who records an attack at Cornwallis, Oregon, in 1916 upon cultivated strawberry and, subsequently (1922), upon wild strawberry in the same State. Later, Smith (1922) in Idaho writes: "The most obvious symptoms of the disease (*A. dipsaci*) on strawberry are enlargement and distortions of the stems, petioles and runners. Commonly the tops of flowering stems, petioles and leaves are swollen, dwarfed and abnormally shaped." Smith, while principally concerned with attack upon red clover, records an instance in which clover and strawberry upon adjacent land were both attacked. In one instance these crops overlapped; nevertheless, he could obtain no evidence that the nematodes could cross from the one to the other. Smith figures attacked strawberry plants and, making due allowance for varietal difference, his description and figures correspond closely with those symptoms observed in Britain.

As opposed to this, Godfrey (1934) describes and figures attacks upon *Fragaria chiloensis*, a wild strawberry, in the Pacific Northwest. His description of the injury caused differs markedly from that of Smith (1922) and, also, he found that he could readily transfer the nematodes from strawberry to red clover seedlings.

Finally, Courtney (1936) records and figures an attack upon strawberry, variety Marshall, following an attacked crop of red clover. Never-

theless, in this instance, the attack on strawberry resembles that described by Smith rather than that by Godfrey. Also, it should be noted that, in this instance, narcissus in the same land apparently escaped injury.

The foregoing accounts suggest that at least two, possibly three, biologic strains of the nematode, each with a different host range, have been observed upon strawberry in the United States. The following records indicate that more than one strain causes very similar injury in Britain.

Occurrence in Britain

(1) *In the variety Madame Lefebvre* (*Pl. XVII, fig.* 1)

In 1934, the attention of the writer was drawn to a peculiar malformation of strawberry plants, variety Madame Lefebvre, occurring abundantly upon a commercial plantation in the Isle of Wight. Various "virus" diseases were present upon plants of other varieties growing in adjacent beds, and it was at first surmised that the malformation might be attributable to such infections. However, it was noted that upon some of the Madame Lefebvre plants, one or more crowns might be affected whilst others remained normal, a somewhat unlikely phenomenon if virus were responsible. Microscopic examination disclosed the invariable presence of *Anguillulina dipsaci* in affected plants. It was ascertained that parents of the plants had come originally from the mainland, and an examination of this parent stock disclosed a less severe but otherwise similar infection. This stock had originally been imported direct from the Continent and as the trouble, at least in 1934, was not found to be widely distributed in the variety elsewhere, it may be suggested that the plants were infected at the time of importation.

From 1934 until the time of writing (December 1937) this nematode infection has been continually under observation and some interesting facts have emerged. It appears that the strain of nematode concerned is a very highly specialized one. On the two commercial holdings on which the incidence has been followed closely, infestation has persisted throughout in the variety Madame Lefebvre, but has not spread to other varieties of strawberry and has not been found upon other cultivated plants or upon weeds. Infested plants removed from the Isle of Wight centre and established in the experimental plots at Reading have continued infested, and have passed infection on to their progeny but have entirely failed to infect strawberries of the varieties Royal Sovereign and Oberschlesien. This has remained true even when the latter varieties have been grown intermingled with infested plants and the whole bed

been allowed to produce runners indiscriminately, Madame Lefebvre runners only contracting an infection. Clover has not yet been subjected to infection and may, of course, prove to be an alternative host of the strain, but in 1935 and 1936 heavily infested Madame Lefebvre plants were grown over narcissus, varieties Soleil d'or and Golden Spur. In no case were the bulbs or foliage attacked or even entered by straying nematodes.

(2) *In the variety Royal Sovereign* (*Pl. XVII, fig.* 2)

Attack on this variety has been found only at Reading and has been referred to briefly by Buddin (1938). It was observed in two situations and, in each instance, the parent plants had been under observation for some time and could be ruled out as not providing a source of infection. Attack occurred in a series of plants grown in boxes and also in plants grown in an experimental plot. In each case it was definitely known that narcissi attacked by *A. dipsaci* had occupied the soil previously and no other probable source of infection could be found. At the outset the plants were entirely crippled by the attack, but in the course of time almost complete recovery took place. Now, upwards of 18 months after the initial infection occurred, symptoms are hard to find although the nematodes have, in fact, persisted in small numbers in some plants.

The inference to be drawn from the above is that at least one narcissus strain of the nematode is able vigorously to attack strawberry, variety Royal Sovereign, but that in the course of time the infestation tends to diminish. It must be recorded that the symptoms induced at the outset of these attacks were identical with those observed in the variety Madame Lefebvre, but in an infection experiment laid down recently, certain of the plants developed typical "Red Plant" symptoms, and further investigation of the problem is proceeding. Nevertheless, it certainly appears to be the case that several biologic strains of *A. dipsaci* can attack strawberry, and that the host ranges of the strains implicated here differ from those recorded from America.

Symptoms of attack

Leaves, petioles and flower stems are invaded. Affected leaves are brittle, curled, usually with the margins bent downwards, more or less crumpled, often somewhat thickened, particularly towards the junction with the petiole, basal portions of the main veins are somewhat thickened and the whole leaf frequently is extremely dwarfed. Petioles and flower stems are more or less thickened and shortened. In extreme cases the petioles are so shortened and the leaves so crumpled that the whole

crown forms a small tight rosette. In popular terms a typically affected leaf and petiole when removed from the plant superficially resembles closely a miniature stick of rhubarb. Although the leaf veins and petioles often exhibit somewhat uneven thickenings there is a marked absence of true "spikels" usually so typical of attack by this nematode and figured on wild strawberry by Godfrey & McKay (1934).

Commonly on a mature plant one or more crowns are badly affected while others may be to all appearances normal. Similarly an affected crown may throw rosette-like runners on thick swollen stolons, whilst other portions of the plant produce normal runners. The symptoms are most apparent in spring, autumn, and winter, and are liable to be masked to a considerable extent during the early summer when plants are growing freely.

ECONOMIC IMPORTANCE OF CONTROL MEASURES

As indicated previously, it appears that attacks by *A. dipsaci* on strawberry are not at present very widespread in this country, but that they occur at all gives cause for concern. Where observed on commercial holdings the depressing effect exercised, not only on the general vigour of the plantation, but also very definitely upon the cropping power of the plants has been apparent. Further, as strawberry plants are invariably propagated by vegetative means, there is every likelihood of infection being distributed elsewhere whenever runners are taken from affected plantations. In practice this cannot be overcome even by careful selection, for unless the runner at the time of planting has a fairly high nematode content, it will be unlikely at that time to exhibit any very marked symptoms of attack.

It is unlikely that plants can be freed from infection by the hot-water treatment. A strawberry plant cannot be relied upon to withstand, at a very maximum, more than 40 min. immersion in water at 110° F. This leaves only a small margin on the time taken to kill the nematode within a leaf or stem and, in practice, the treatment would probably be only partially successful. A small experiment carried out in 1936 confirmed this impression, surviving nematodes quickly again building up a large population within the plants.

In effect, with our present knowledge it can only be suggested that once the presence of *A. dipsaci* is diagnosed in a strawberry plantation, that plantation should on no account be used for runner production. Unless the attack is severe there is no great objection to leaving the plants in for the rest of their useful fruiting life, provided that precautions

against spreading infection are taken. At the termination of this period, some other crop should be substituted in the land for a few seasons.

As has been shown, if the attacked crop is the variety Madame Lefebvre the substitution of another variety of strawberry might meet the case. Unless, however, this is imperative it would be as well to select a less likely host, bearing in mind that clover and narcissus have, in some instances, proved acceptable alternatives to the nematode. In any case, predominant weeds in the locality should be carefully scrutinized as being possible host plants in which the nematodes might carry over until such time as strawberries are again planted in the land.

Summary

1. Attention is drawn to the occurrence of *Anguillulina dipsaci* on strawberry in Britain and reference is made to literature relating to such occurrence in the United States of America.

2. Recorded occurrences in Britain are discussed in some detail and reference is made to the host range of the strains of nematode implicated.

3. Symptoms of attack are described.

4. Control measures are discussed, the principal suggestion being that on no account should runners be taken for propagation from infected plantations.

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EXPLANATION OF PLATE XVII

Fig. 1. Madame Lefebvre, two-year-old plant. Attacked by *Anguillulina dipsaci* (strawberry strain). Contrast normal portion of plant with crumpled crown on right.

Fig. 2. Royal Sovereign, maiden plant. Attacked by *Anguillulina dipsaci* (narcissus strain). Contrast relatively healthy peripheral leaves with those around growing point.

(*Received* 14 *December* 1937)

Photo A. Sherval

Fig. 1.

Photo A. Sherval

Fig. 2.

HODSON.—THE STEM AND BULB EELWORM, *ANGUILLULINA DIPSACI* (KUHN), IN STRAWBERRY IN BRITAIN (pp. 406–410)

THE PROBLEM OF THE EVALUATION OF ROTENONE-CONTAINING PLANTS

IV. THE TOXICITY TO *APHIS RUMICIS* OF CERTAIN PRODUCTS ISOLATED FROM DERRIS ROOT

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(With 2 Text-figures)

CONTENTS

Introduction

In Parts II and III of this series of papers (Martin & Tattersfield, 1936; Tattersfield & Martin, 1936) it was shown that there was a difference in the chemical make-up of the resins derived from the roots of the two species of *Derris*, *D. elliptica* and *D. malaccensis*. Thus the resins of the

former gave rise to little toxicarol when an ether solution was treated with alcoholic potash, whereas those of the latter were shown by chemical and polarimetric tests to be rich in a precursor which yielded toxicarol on subsequent treatment with alcoholic potash. This precursor was isolated in crystalline form (Tattersfield & Martin, 1937) and occurs in considerable amount in the Sumatra-type root; indeed, such roots are far richer in this product than they are in rotenone. Recently, also, Cahn & Boam (1935) have isolated from the Sumatra-type root a colourless crystalline derivative, which they have named sumatrol, and which they have shown to possess toxic properties. It is a matter of importance for evaluation purposes to know with some degree of accuracy how rotenone compares in toxicity with some of these isolated products, particularly with toxicarol, its precursor, with sumatrol and also with the resins derived from both *D. elliptica* and *D. malaccensis* when these have been freed as far as possible from crystalline material. The latter process must not be a drastic one, for there is now considerable evidence that certain of the crystalline products isolated from derris root have undergone change in the process of separation.

In the present work, two resins are dealt with, one derived from *D. elliptica* and one from *D. malaccensis* (Sumatra-type root). In the former the purified rotenone content was 8·9 %. In the process of separation no additional rotenone was added to aid its separation. It is a matter of doubt whether any of the processes at present employed for the separation of rotenone assures the removal of all present. The data given here, therefore, are necessarily a preliminary to further work on this subject.

The insecticidal tests were carried out by our usual procedure, using adult apterous females of *Aphis rumicis*. The experiments were made in fivefold replication, using five batches of ten insects for each concentration tested. The method adopted for comparing toxicity is based upon the work of Bliss (1935) and of Irwin (1937), a detailed example being given by Mr W. G. Cochran in the Appendix.

As the experiments have been spread over two seasons and the average susceptibility of the insects was not the same throughout the period, tests done on different dates cannot be expected to give the same absolute figures, but the comparative results for the toxicities relative to rotenone of different preparations of the toxicarol precursor are of the same order, the differences found not being significant.

The results indicate that of the known unaltered active principles of *Derris*, rotenone is the most toxic and therefore its determination in samples of derris root will always be necessary. As, however, both

the resins and such active principles as the toxicarol precursor and sumatrol may be present in certain strains of root in higher concentrations than rotenone, they cannot be neglected in assessing toxicity with any degree of accuracy. In addition, it seems doubtful whether the same toxic value can be placed upon the constituents left after the separation of rotenone in different strains of root.

EXPERIMENTAL

Preparation of the toxicarol precursor

In footnotes to Parts II and III of this series (*loc. cit.*) mention was made of an optically active crystalline compound that had been isolated from the ether solution of the Sumatra-type root by extraction with caustic potash. On the addition of small amounts of dilute caustic potash (2 %) to the ether solution of the Sumatra-type resin, a colloidal material was first extracted followed by a dark red clear solution on the addition of a further quantity of dilute alkali. On subsequently treating the ethereal layer with 5 % caustic potash, a copious bright yellow precipitate was deposited in the alkaline layer. On filtering, dispersing in water and acidifying in the presence of ether, a resin rich in toxicarol precursor was taken up by the ether. The ether solution after drying over anhydrous sodium sulphate deposited some yellow crystals which were filtered off and discarded. After concentrating and cooling in the refrigerator for some days a large mass of micro-crystalline matter was deposited. Repeated crystallizations from acetic acid or ethyl acetate raised the melting-point to 99° C. This product we have termed toxicarol precursor (potash separated).

As it is well known that the products derived from derris root are susceptible to the effects of alkali, it was considered advisable to attempt the separation of this product by direct means. This was finally effected by allowing a concentrated ethyl acetate solution of a Sumatra-type resin to evaporate slowly in the refrigerator. A small crop of crystals was obtained in this way, which proved of great value in seeding out larger quantities of the crude precursor from more dilute solutions of the resin. With material for seeding purposes, no difficulty presents itself in preparing this compound in a crystalline form. The controlled precipitation of the concentrated ether solution of the crude crystals with petroleum ether separated out first a darker coloured impure deposit, a further crop of relatively impure crystalline matter separating on cooling the supernatant liquid in the refrigerator after which the solution was concentrated in partial vacuum, cooled and the crystalline matter recrystal-

lized from ethyl acetate. The whole process was repeated several times. Repeated crystallizations raised the melting-point from 96 to 103° C. A brief account of these two derivatives has been published elsewhere (Tattersfield & Martin, 1937) but it is probable that even the material of higher melting-point is not completely pure.[1] With the material so far available, the following data have been accumulated:

Toxicarol precursor (potash separated).

Melting-point 99–100° C.

$[\alpha]_D^{20} = -68{\cdot}8°$ in benzene, concentration 4·0536 g. per 100 ml.

$[\alpha]_D^{20} = -71{\cdot}2°$ in benzene, concentration 2·0268 g. per 100 ml.

$[\alpha]_D^{20} = +41{\cdot}5°$ in ethyl alcohol, concentration 1·0008 g. per 100 ml.

Toxicarol precursor (direct separation).

Melting-point 96° C.

$[\alpha]_D^{20} = -66{\cdot}8°$ in benzene, concentration 4·0009 g. per 100 ml.

$[\alpha]_D^{20} = -70{\cdot}9°$ in benzene, concentration 2·0044 g. per 100 ml.

Melting-point 95° C.

$[\alpha]_D^{20} = -66{\cdot}8°$ in benzene, concentration 4·0184 g. per 100 ml.

$[\alpha]_D^{20} = -69{\cdot}7°$ in benzene, concentration 2·0092 g. per 100 ml.

Melting-point 102–103·5°.[1]

$[\alpha]_D^{20} = -67{\cdot}6°$ in benzene, concentration 4·3168 g. per 100 ml.

$[\alpha]_D^{20} = +44{\cdot}8°$ in ethyl alcohol, concentration 1·0044 g. per 100 ml.

$[\alpha]_D^{20} = +41{\cdot}5°$ in ethyl alcohol, concentration 1·0136 g. per 100 ml.

$[\alpha]_D^{20} = +41{\cdot}8°$ in ethyl alcohol, concentration 0·7174 g. per 100 ml.

Change in sign of rotation

The precursor separated either by potash treatment or by direct crystallization shows the change over in the rotation of its benzene solution when caustic potash in methyl alcohol is added. The subsequent decline in rotation proceeds at approximately the same speed as that found for the Sumatra-type resin and for the potash-extracted resin, when the amount of alcohol added and the temperature of the reaction are the same. Progressive darkening of the solution renders readings of the instrument very difficult after some time and finally impossible, but as far as could be judged the fall in rotation does not proceed to zero,

[1] Cahn (private communication) has confirmed the presence of this precursor in the resins of the Sumatra-type root, and has given much attention to its purification and constitution. The purification of this derivative is one of great difficulty and purity cannot be judged by melting-point considerations. Our product, in his view, contains almost certainly a proportion of sumatrol and the true rotation of the precursor is definitely lower than the figures given.

possibly indicating the presence of some other material. When the final reading is deducted, the rotations follow an approximately unimolecular curve. The coefficient $k=\frac{1}{t_2-t_1}\log\frac{a}{a-x}$, however, falls off slowly, but during the middle period of the reaction is nearly constant.

The following is a summary of the results:

Toxicarol precursor (potash separated).

1 g. equivalent of caustic potash in methyl alcohol added, the molecular weight being regarded as the same as toxicarol.

Concentration of benzene solution 2·2027 g. per 100 ml.

Proportion of benzene solution : methyl alcohol :: 5 : 2.

$[\alpha]_D^{20}$ for benzene solution $= -71{\cdot}2°$.

$[\alpha]_D^{20}$ after adding methyl alcohol $= -31{\cdot}7°$.

On adding 1 g. equivalent methyl alcoholic potash:

$[\alpha]_D^{20}$ after 2 min. $= +302{\cdot}5°$.

$[\alpha]_D^{20}$ after 15 min. $= +236°$.

$[\alpha]_D^{20}$ after 120 min. $= +120°$.

$[\alpha]_D^{20}$ after 360 min. $= +56°$.

After 23 hr. an accurate reading could not be taken but $[\alpha]_D^{20}$ was not lower than $+52°$.

Mean k after deducting $[\alpha]_D^{20}$ for $t=360$ min. for intervals

$$t=13 \text{ min. to } t=118 \text{ min.} = 0{\cdot}0095 \text{ to log base } 10$$
$$= 0{\cdot}022 \text{ to log base } e.$$

Toxicarol precursor by direct separation.

1 g. equivalent of caustic potash added in methyl alcohol.

Concentration of benzene solution 2·0092 g. per 100 ml.

Proportion of benzene solution : methyl alcohol :: 5 : 2.

$[\alpha]_D^{20}$ for benzene solution $= -69{\cdot}7°$.

$[\alpha]_D^{20}$ after adding methyl alcohol $= -33{\cdot}4$.

On adding methyl alcoholic caustic potash:

$[\alpha]_D^{20}$ after 2 min. $= +285°$.

$[\alpha]_D^{20}$ after 15 min. $= +222{\cdot}3°$.

$[\alpha]_D^{20}$ after 120 min. $= +55{\cdot}7°$.

$[\alpha]_D^{20}$ after 360 min. $= +35{\cdot}5°$.

$[\alpha]_D^{20}$ after 1560 min. $= +32{\cdot}2°$.

Mean k after deducting $[\alpha]_D^{20}$ for $t=1560$ min. for intervals

$$t=13 \text{ min. to } t=88 \text{ min.} = 0{\cdot}0093 \text{ to log base } 10 = 0{\cdot}0021 \text{ to log base } e.$$

The data from two examples out of several tests are plotted in Fig. 1, those for the potash separated precursor (M.P. 99° C.) being shown in

section A, and for the compound separated directly (M.P. 96–98° C.) in section B.

The results are expressed in each section in two ways. The times which have elapsed from adding the caustic potash in methyl alcohol to the respective benzene solutions are plotted against (*a*) the specific rotation, (*b*) the logarithms of the figures given by deducting the last observable specific rotation from each of the others. There is a slight difference in the results for the two products probably due either to differences in purity, or to difficulties found in controlling the temperature, particularly in the initial stages of the reaction.

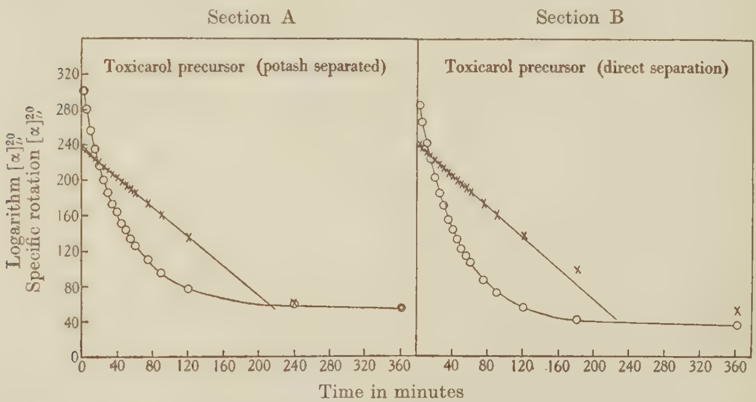

Fig. 1. Decline in specific rotation of toxicarol precursor in benzene solution after the addition of caustic potash in methyl alcohol. ○ ○ Specific rotations $[\alpha]^{20}_D$; × × $\log_{10}$ specific rotations $[\alpha]^{20}_D$.

It is clear, however, from the close parallelism of the semi-logarithmic data graphed in the two sections of Fig. 1 that they each contain a very high proportion of the same active constituent.

Tests were also carried out with solutions in ethyl alcohol of the compound isolated by direct crystallization, and melting 102–103·5° C. In one case 1 g. equivalent and in the other 4 g. equivalents of caustic potash in ethyl alcohol were added. Owing to the rapidity of the fall in optical activity and the relatively rapid darkening of the solutions the results were not very satisfactory. The results are summarized below.

First concentration of the alcoholic solution 1·0044 g. per 100 ml. $[\alpha]^{20}_D = +44{\cdot}8°$.

Concentration, after adding ethyl alcohol equal in amount to that introduced with the caustic potash, $=0{\cdot}7174$ g. per 100 ml. $[\alpha]^{0} = +41{\cdot}8°$

On adding 1 g. equivalent of caustic potash in methyl alcohol.
$[\alpha]_D^{20}$ after 2 min. = +166°.
$[\alpha]_D^{20}$ after 5 min. = +89°.
$[\alpha]_D^{20}$ after 90 min. = +56°.

On adding 4 g. equivalent of caustic potash in same volume of alcohol $[\alpha]_D^{20}$ after 1 min. = +293°.
$[\alpha]_D^{20}$ after 2 min. = +248°.
$[\alpha]_D^{20}$ after 5 min. = +157°.
$[\alpha]_D^{20}$ after 114 min. = +46°.

The value $\frac{1}{t_2-t_1}\log\frac{a}{a-x}$ was not constant.

INSECTICIDAL TESTS

Preparation of spray fluids

Rotenone. Rotenone was separated as the carbon tetrachloride complex and recrystallized several times from absolute alcohol, the product being finally dried in a vacuum desiccator. A weighed quantity was dissolved in absolute alcohol and made up to a known concentration. Dilutions were made with 0·5% saponin solution and each dilution adjusted to contain the same proportion of alcohol. The dilution for each concentration used was made just prior to spraying, not more than a few minutes elapsing between the addition of the saponin solution and the completion of the five replications. Thus the risk of aggregation of particles was minimized and no separation of a deposit was noted.

Toxicarol precursor preparations. These included: (1) The micro-crystalline material isolated from the Sumatra-type resin, by extracting the ether solution of the latter with caustic potash and recovering the free compound from the potash-precipitated material by acidification and extraction. (2) The crystals obtained from the latter by crystallization from glacial acetic acid. It was a matter of some difficulty to free the product from the last traces of acetic acid; it was therefore thoroughly washed with cold alcohol, and the last traces of solvent taken off *in vacuo*. (3) The micro-crystalline crude product isolated was frequently recrystallized from ethyl acetate. (4) The precursor separated by direct crystallization from a sample of the Sumatra-type resin was subjected to the process of purification outlined on p. 413 and finally recrystallized several times from ethyl acetate (M.P. 102–103·5° C.).

For the insecticide trials, a solution in alcohol of known strength was diluted in precisely the same way as in the case of rotenone.

Inactive toxicarol. The precursor dissolved in ethyl alcohol was refluxed with a small amount of aqueous caustic potash, allowed to stand for an hour, the deposit filtered off, washed with boiling absolute alcohol, dried at 60–80° C. for half an hour and recrystallized from acetic acid. As toxicarol is relatively insoluble in alcohol, it was dissolved in benzene and emulsions of different concentrations were prepared by the addition of 0·5 % saponin just before spraying. Under these conditions the product showed little or no toxicity at 0·5 % concentration.

Sumatrol. The crystals were dissolved in acetone, and the appropriate dilutions made just before spraying with 0·5 % saponin solution in water, the amount of acetone being adjusted to the same content in each.

Resin from the Sumatra-type root freed as far as possible from crystallizable material. The ether extract of a large quantity of the root was transferred to a large separating funnel and extracted successively with four lots of aqueous potash (5 %). The extracted ether solution was thoroughly washed with water and dried over sodium sulphate, the ether removed and the residue dissolved in carbon tetrachloride and cooled in a refrigerator for 4 days. The rotenone crystallized and was filtered off. The solvent in the filtrate was taken off under reduced pressure with warming, in a vacuum desiccator, and finally at 100° C. under reduced pressure. Dilutions for spraying purposes were made from an alcohol solution of known content precisely as in the case of rotenone.

Resin from Derris elliptica. The ether extract was dissolved in carbon tetrachloride and cooled in a refrigerator for many weeks, the rotenone filtered off, and the filtrate cooled again. After the separation of a further small amount of crystalline matter, the solvent was taken off as with the Sumatra-type resin and dilutions for spraying purposes made as for rotenone from an alcohol solution of known content of resin.

Results

Adult apterous females of *Aphis rumicis* were used as test subjects with the usual technique for spraying and assessing the results. Ten insects at a time were sprayed and five replications were made. The insects after being sprayed were kept in muslin-covered tubes in a dark room of relatively constant temperature, examined after 2 days (44 hr.), and separated into five categories: (1) the unaffected, (2) those slightly affected, (3) the seriously paralysed (B), (4) the moribund (M), and (5) the apparently dead (D). Since in the last three categories there is rarely, if ever, any recovery from the effects of these compounds, the number

Table I

Relative potencies of rotenone and certain constituents of Derris

Insect used *Aphis rumicis*. Sprayed in fivefold replication 10 insects at a time. b_c = mean slope of regression lines, $V(b_c)$ its variance, $\Sigma(w_1)$ and $\Sigma(w_2)$ = sum of weights, s_M^2 = variance of M.

Relative potencies (antilog M)

1. $M=\bar{x}_1-\bar{x}_2+\dfrac{\bar{y}_2-\bar{y}_1}{b_c}$

2. $\text{S.E.}_M=\sqrt{s_M^2}=\sqrt{\dfrac{1}{b_c^2}\left\{\dfrac{1}{\Sigma(w_1)}+\dfrac{1}{\Sigma(w_2)}+\dfrac{(\bar{y}_2-\bar{y}_1)^2}{b_c^2}V(b_c)\right\}}$

3. S.E. of relative potency $=\log_e 10\times\text{antilog } M\times s_M$

Conc. mg./litre	Log conc. x	Paralysed and dead insects % allowing for control	Probits y	No. of insects used	Relative potencies
Series 1. Section A of Fig. 2.					
Toxicarol precursor (potash separated) (microcrystalline):					
400	2·602	97·9	7·034	49	
200	2·301	67·3	5·448	50	
100	2·000	14·9	3·959	48	$M=1·1638$
75	1·875	4·0	3·249	50	$\text{S.E.}_M=\pm 0·0358$
					$\dfrac{\text{Potency of rotenone}}{\text{Potency of toxicarol precursor}}=\text{antilog } M=14·6$
Rotenone:					
40	1·602	100	8·232*	51	S.E. of relative potency $=\pm 1·2$
20	1·301	90	6·282	51	
10	1·000	39·5	4·734	49	
5	0·699	4·7	3·325	45	
Control		2·1		47	
Series 2. Section B of Fig. 2.					
Toxicarol precursor (potash separated) (crystallized from acetic acid):					
400	2·602	77·5	5·755	51	
300	2·477	66·7	5·432	50	
200	2·301	30·5	4·490	51	$M=1·1863$
150	2·176	6·25	3·466	50	$\text{S.E.}_M=\pm 0·0287$
					$\dfrac{\text{Potency of rotenone}}{\text{Potency of toxicarol precursor}}=\text{antilog } M=15·4$
Rotenone:					
30	1·477	89·4	6·248	49	S.E. of relative potency $=\pm 1·0$
20	1·301	72·9	5·610	50	
15	1·176	29·8	4·470	49	
Control		4·0		50	
Series 3. Section C of Fig. 2.					
Toxicarol precursor (direct separation), M.P. 102–103·5:					
					$M=1·1629$
301	2·479	97·9	7·033	50	$\text{S.E.}_M=\pm 0·0398$
201	2·303	74·4	5·656	50	$\dfrac{\text{Potency of rotenone}}{\text{Potency of this sample of precursor}}=\text{antilog } M=14·6$
100·4	2·002	25·2	4·332	50	S.E. of relative potency $=\pm 1·33$
Toxicarol precursor (potash separation) (crystallized from ethyl acetate):					
					$M=1·1137$
300	2·477	95·7	6·717	50	$\text{S.E.}_M=\pm 0·0420$
200	2·301	80·8	5·870	50	$\dfrac{\text{Potency of rotenone}}{\text{Potency of this sample of precursor}}=\text{antilog } M=13·0$
100	2·000	37·2	4·673	51	S.E. of relative potency $=\pm 1·26$

* Computed value.

Table I (*cont.*)

Relative potencies (antilog M)

1. $M=\bar{x}_1-\bar{x}_2+\dfrac{\bar{y}_2-\bar{y}_1}{b_c}$

2. s.e.$_M=\sqrt{s_M^2}=\sqrt{\dfrac{1}{b_c^2}\left\{\dfrac{1}{\Sigma(w_1)}+\dfrac{1}{\Sigma(w_2)}+\dfrac{(\bar{y}_2-\bar{y}_1)^2}{b_c^2}V(b_c)\right\}}$

3. s.e. of relative potency $=\log_e 10\times$ antilog $M\times s_M$

Conc. mg./litre	Log conc. x	Paralysed and dead insects % allowing for control	Probits y	No. of insects used	Relative potencies (antilog M)
Series 3 (*cont.*)					
Sumatrol:					$M=1{\cdot}1179$
404	2·606	100	7·338*	50	s.e.$_M=\pm0{\cdot}0454$
302	2·480	92·7	6·454	50	$\dfrac{\text{Potency of rotenone}}{\text{Potency of sumatrol}}=$ antilog $M=13{\cdot}1$
201	—	48·8†	—	50	
100·6	2·002	37·8	4·689	49	s.e. of relative potency $=\pm1{\cdot}37$
Rotenone:					
31	1·491	100	7·506	50	
20·7	1·316	91·5	6·372	50	
10·4	1·017	53	5·075	50	
5·2	0·716	19·3	4·133	49	
Control		6·4		47	
Control for sumatrol		18		50	
Series 4. Section D of Fig. 2.					
Sumatra-type resin (separated from rotenone and toxicarol precursor):					
300	2·477	100	7·966*	49	
200	2·301	100	7·035*	48	
100	2·000	35·4	4·626	49	$M=0{\cdot}800$
80	1·903	8·6	3·634	48	s.e.$_M=0{\cdot}0247$
60	1·778	9·1	3·665	46	$\dfrac{\text{Potency of rotenone}}{\text{Potency of this resin}}=$ antilog $M=6{\cdot}3$
					s.e. of relative potency $\pm0{\cdot}36$
Rotenone:					
20	1·301	61·7	5·298	48	
15	1·176	33·4	4·571	49	
Control		2·0			
Series 5. Section E of Fig. 2.					
Derris elliptica resin (W. 186) (separated from rotenone):					
100·5	2·002	100	7·679*	50	
80·4	1·905	100	7·190*	50	
60·3	1·780	84·1	5·999	50	$M=0{\cdot}5515$
40·2	1·604	50	5·000	50	s.e.$_M=\pm0{\cdot}0311$
					$\dfrac{\text{Potency of rotenone}}{\text{Potency of this resin}}=$ antilog $M=3{\cdot}6$
Rotenone:					s.e. of relative potency $=\pm0{\cdot}26$
20·7	1·316	97·7	6·995	50	
10·4	1·017	35·1	4·617	49	
Control		12·0			
Series 6. Section F of Fig. 2.					Results omitting value in brackets for rotenone
D. elliptica resin (W. 186) (separated from rotenone):					
100	2·000	100	7·696*	47	$M=0{\cdot}5653$
80	1·903	100	7·296*	49	s.e.$_M=\pm0{\cdot}03225$
60	1·778	91·2	6·353	49	$\dfrac{\text{Potency of rotenone}}{\text{Potency of this resin}}=$ antilog $M=3{\cdot}7$
40	1·602	55·9	5·148	46	
20	1·301	15·9	4·001	47	s.e. of relative potency $=\pm0{\cdot}27$
Rotenone:					Results taking value in brackets for rotenone
31	1·491	100	8·168*	49	$M=0{\cdot}5896$
20·7	1·316	97·9	7·033	50	s.e.$_M=\pm0{\cdot}0284$
(15·5	1·190	95·6	6·706	49)	$\dfrac{\text{Potency of rotenone}}{\text{Potency of this resin}}=$ antilog $M=3{\cdot}9$
10·4	1·017	56·4	5·161	49	
7·8	0·892	39·6	4·736	39	s.e. of relative potency $=\pm0{\cdot}25$
Control		6·4			

* Computed value. † Value omitted from calculation as it introduces heterogeneity.

falling within them were listed together for judging toxicity ($B+M+D$). Occasionally during spraying an insect was lost or in the examination a diseased insect had to be eliminated, accounting for occasional discrepancies from the figure 50 shown in the tables.

The data are shown in Table I. The concentrations and their logarithms (x), the percentage of seriously affected insects with the corresponding probits and the actual numbers of insects used for assessment and determining the "weights" to be allotted are given. In addition, the result of each experiment is shown in the last column. In

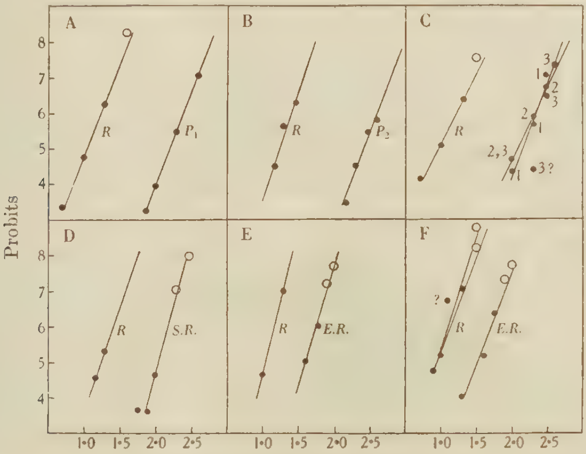

Fig. 2. Probits plotted against log concentrations for rotenone, and various constituents of derris root. ○ computed, R rotenone, 1 toxicarol precursor, direct separation, 2 toxicarol precursor, potash separation, 3 sumatrol, $S.R.$ rotenone-freed Sumatra resin, $E.R.$ rotenone-freed *D. elliptica* resin, P_1 micro-crystalline precursor separated from ether, P_2 recrystallized acetic acid.

Fig. 2 the probits are plotted against the logarithms of the concentrations, the lines drawn through the points being calculated. There was on the whole little difference in position between the free-hand lines used for assessing the "weights" described by Bliss (1935) and the calculated lines, and since the scale of plotting is small, only the latter are shown in order to avoid confusion.

The method of statistical analysis is somewhat laborious, but has proved in our experience to be of considerable value. Its use and particularly the test for the heterogeneity makes it possible to eliminate unreliable values, due to unavoidable imperfections in the process of

carrying out a large-scale laboratory spraying experiment. Such points have been eliminated from the data. In addition, it was noted that above certain levels of concentration there was a decline in toxicity. We have observed this effect with derris preparations over a period of years. The most valid explanation appears to be that the result is due to aggregation of particles in the emulsions or possibly in some cases to the formation of crystals above an optimum size, since it was shown by Fryer *et al.* (1923) that the toxicity of rotenone depended upon the size of the crystals. Therefore, in order to eliminate this effect as far as possible, dilutions of the concentrated extracts were made in every case just before spraying.

Bliss (1935) has also demonstrated that frequently in these types of data there is, for small probit values, a departure from the general linearity obtained at higher concentrations. This was not a noticeable feature of our data, but in one or two cases at the lowest concentrations tested there was some indication of this break and these values have not been included in the analyses.

In one case (Table I, series 3 and Fig. 2, section 3), heterogeneity was introduced into the data by one determination of toxicity unquestionably faulty and this was eliminated. The point is shown with a query mark. In section F, Fig. 2, a point of questionable validity also appears, but as it does not introduce heterogeneity we have calculated the relative potencies in this case, both by leaving it out, and taking it into account. The results, however, are not significantly different, and both regression lines are given in section F.

In section C the slope of the line for the toxicarol precursor (direct separation) is slightly steeper than that representing the potash-separated precursor and sumatrol, but as neither line differs significantly from that of rotenone in parallelism this difference may be regarded as of no importance. The data for sumatrol are not entirely satisfactory, one experimental value being obviously an under-estimate. We do not wish therefore to put any great stress upon the determination of the relative potency of this compound, as owing to its low solubility in alcohol, acetone was used in the preparation of the spray and the control test, in which an equivalent amount of acetone was used, led to the relatively high figure of 18 % of paralysed insects. The results, however, indicate that sumatrol is of the same order of toxicity to *Aphis rumicis* as the toxicarol precursor. Toxicarol itself when sprayed in benzene emulsions had little or no toxicity at 0·5 % concentration.

The relative toxicities given by comparisons at the median lethal

doses approximately computed from the free-hand regression lines, together with those determined statistically are shown in Table II.

Table II

Comparison of estimates of relative potencies computed from free-hand probit-log-concentration lines at probit 5·0 *and by statistical analysis*

Results expressed as $\frac{\text{Potency of rotenone}}{\text{Potency of substance tested}}$.

Section in Fig. 2	Rotenone compared with	Log conc. of rotenone at probit 5·0	Log conc. of substance tested at probit 5·0	Difference	Relative potency computed from free-hand lines at probit 5·0 antilog of difference	Relative potency from statistical computation
A	T. precursor (KOH separated microcrystalline)	1·045	2·210	1·165	14·6 : 1	14·6 : 1
B	T. precursor (KOH separated crystallized acetic acid)	1·235	2·405	1·170	14·8 : 1	15·4 : 1
C	T. precursor (KOH separated crystallized ethyl acetate)	0·978	2·085	1·107	12·8 : 1	13·0 : 1
C	T. precursor (direct separation)	0·978	2·165	1·187	15·4 : 1	14·6 : 1
C	Sumatrol*	0·978	2·150	1·172	14·9 : 1	13·1 : 1
D	Sumatra-type resin	1·250	2·060	0·810	6·5 : 1	6·3 : 1
E	*Derris elliptica* resin (1)	1·065	1·610	0·545	3·5 : 1	3·6 : 1
F	*Derris elliptica* resin (2)†	0·965	1·525	0·560	3·6 : 1	3·7 : 1

* A discrepant point taken in. † A discrepant point omitted.

The figures from the free-hand lines were obtained by taking the lines used for computing the weights in the statistical analyses and reading off the respective log concentrations corresponding to 5·0 probits, subtracting and reading the antilog. The differences between the relative potencies by the two methods are not great, and are not significantly different from each other. The shorter method, however, does not permit determination of the standard error and unless the points through which it is drawn are disposed in such a way as to be easily fitted by eye, a considerable degree of error may result. Such a method, however, does give a very useful preliminary estimate and in many cases may be all that is required.

It may be noted that the potencies of the samples of the toxicarol precursor are not significantly different from each other. Sumatrol also, in our experiments has the same order of toxicity to *A. rumicis* as the toxicarol precursor, and although our samples of the precursor may

contain a certain proportion of sumatrol, the indications are that this order of toxicity would be shown by the pure precursor unless a highly potent but as yet unrecognized compound is also present.

The rotenone and toxicarol-free resin of the Sumatra-type root and the rotenone-free resin of *Derris elliptica* are more toxic than either the toxicarol precursor or sumatrol, but the resin derived from the Sumatra-type is less toxic than that derived from *D. elliptica*. Rotenone was six times as toxic as the Sumatra-type resin and four times that derived from *D. elliptica*. Rotenone was not in either case added as an aid to its own separation, but it is reasonable to believe that the presence of rotenone in the resins would, if anything, increase toxicity, and the deduction can be drawn that the suggestion of Jones & Smith (1936) to evaluate the residual rotenone-free resins as possessing half the toxic value of rotenone is scarcely justified as far as *Aphis rumicis* is concerned. Jones & Smith, however, used house flies for the determination of their toxicities, and it is possible that the degree of difference may depend on the test-subject used.

Conclusions

Previous papers in this series (*loc. cit.*) have indicated a difference in the chemical constitution of the resins extracted from roots of *Derris elliptica* and *D. malaccensis*. In this paper it is shown that a crystalline derivative giving rise to toxicarol on treatment with alcoholic potash and which may be regarded as its precursor, is largely responsible for the chemical and physical properties which differentiate the Sumatra-type and *D. malaccensis* resins from those of *D. elliptica*. This compound is readily isolated in large amounts from the potash extraction of ether solutions of Sumatra-type resin or by direct crystallization from the latter, and is responsible for the characteristic change in sign of rotation of the benzene solutions of the resins of *D. malaccensis* on addition of alcoholic potash. It is laevo-rotatory in benzene and dextro-rotatory in alcohol. After the addition of caustic potash in alcohol to either its benzene or alcoholic solutions, the dextro-rotatory power declines with time.

The first preparations by both the above processes melted in the neighbourhood of 97–100° C. The melting-point of the directly separated compound has been raised by repeated crystallizations and precipitations using various solvents to the neighbourhood of 103° C., but the product may still contain allied compounds.

Rotenone is fourteen to fifteen times as toxic as our present preparations of toxicarol precursor. The Sumatra-type resin from which both the precursor and rotenone have been separated is also more toxic, nevertheless the precursor in our tests possesses much more insecticidal power than inactive toxicarol, and in the Sumatra-type root almost certainly contributes in a large measure to its toxicity to insects.

This paper is mainly devoted to the description of toxicity tests made with this compound or, perhaps, complex of compounds, and with the resins freed from rotenone as far as possible without the addition of the latter compound to aid its own separation, rotenone being used as a standard of comparison. An experiment carried out with sumatrol is also included. An Appendix by Mr W. G. Cochran describes the most suitable and least laborious statistical technique applicable to the analysis of our results and to the determination of their significance. A rough method of comparing toxicities by reading directly from the probit-log concentration lines was also employed. We find that the toxicities to *Aphis rumicis* of the toxicarol precursor and of sumatrol are of the same order, about one-thirteenth to one-fifteenth as toxic as rotenone, whereas the resin of the Sumatra-type root is about one-sixth and that of *Derris elliptica* about one-fourth as toxic as rotenone.

These data suggest that one factor for purposes of evaluation cannot be applied to all classes of root, irrespective of their derivation. Thus, it would appear at present unjustifiable to assume that the resins of different varieties, after the separation of rotenone by present methods, have a toxicity of a definite and fixed order, or, until further work has been done on the relationship between the rotation of the various naturally occurring active principles and their toxicities, that a determination of the rotations of extracts derived from different varieties of root will give a valid assessment of their insecticidal value.

We wish to express our indebtedness to the Cooper Technical Bureau for a gift of sumatrol, to Dr R. S. Cahn for permission to publish a short private communication, and to Mr W. G. Cochran for statistical help and advice.

APPENDIX

By W. G. COCHRAN

The following is a detailed example of the computation necessary for determining the relative potencies. The figures are taken from Table I, series 1 (p. 419).

	Log conc. x	Probits y	Weight w	wx	wy
Rotenone	1·602	8·232	0·918	1·470636	7·556976
	1·301	6·282	16·269	21·165969	102·201858
	1·000	4·734	30·429	30·429	144·050886
	0·699	3·325	8·100	5·6619	26·9325
		Total	55·716	58·727505	280·742220
Toxicarol precursor	2·602	7·034	6·958	18·104716	48·942572
	2·301	5·448	29·550	67·994550	160·988400
	2·000	3·959	19·968	39·936	79·053312
	1·875	3·249	10·400	19·5	33·789600
		Total	66·876	145·535266	322·773884

Notes		Rotenone (r)	Toxicarol precursor (p)
(i)	$\bar{x}=\frac{\Sigma\,(wx)}{\Sigma w}$	1·054051	2·176196
	$a=\bar{y}=\frac{\Sigma\,(wy)}{\Sigma\,(w)}$	5·038808	4·826453
(ii)	$A=XX=\Sigma\,(wx^2)-\bar{x}\Sigma\,(wx)$	2·3778	3·2852
	$XY=\Sigma\,(wxy)-\bar{x}\Sigma\,(wy)$	12·0310	16·8258
	$YY=\Sigma\,(wy^2)-\bar{y}\Sigma\,(wy)$	61·1224	86·2283
	(Slope of regression line) $b=\frac{XY}{XX}$	5·0597	5·1217
	$\chi^2=YY-b\,(XY)$	0·249	0·052

χ^2 for $n=n'-2=2$ and $P=0{\cdot}05$ is 5·991, $\therefore$ data may be regarded as not heterogeneous

(iii)	Variance of a: $V(a)=1/\Sigma\,(w)$	0·01795	0·01495
	Variance of b: $V(b)=1/A=\frac{1}{XX}$	0·4206	0·3044

Test for parallelism:

$$\chi_b^2=\frac{(b_r-b_p)^2}{\frac{1}{A_r}+\frac{1}{A_p}}=0{\cdot}005$$

For $n=1$, $P=0{\cdot}05$, $\chi^2=3{\cdot}481$, departure of lines from parallelism not significant

Determination of relative potencies:

$$M=\log\left(\frac{\text{potency of rotenone}}{\text{potency of toxicarol precursor}}\right)=\bar{x}_p-\bar{x}_r+(\bar{y}_r-\bar{y}_p)/b_c$$

Mean slope of combined lines: $b_c=\{(XY)_r+(XY)_p\}/\{(XX)_r+(XX)_p\}=5{\cdot}0957$

$$V\,(b_c)=\frac{1}{A_r+A_p}=\frac{1}{(XX)_r+(XX)_p}=0{\cdot}17658$$

(iv) $M=1{\cdot}1221+0{\cdot}04167=1{\cdot}1638$

Relative potency: Antilog $M=14{\cdot}58$

(v) *Standard error of M (s_M):*

$$s_M=\sqrt{V\,(M)}=\frac{1}{b_c}\sqrt{\left[1/\Sigma\,(w_r)+1/\Sigma\,(w_p)+\frac{(\bar{y}_r-\bar{y}_p)^2}{b_c^2}\,V\,(b_c)\right]}=\pm 0{\cdot}03576$$

(vi) *S.E. of relative potency:* $\log_e 10\times\text{antilog}\,M\times s_M=2{\cdot}3026\times 14{\cdot}58\times 0{\cdot}0358=\pm 1{\cdot}20$

The object of the calculation is to obtain, with the least possible labour, the value of M correct to three decimal places. The specimen calculation includes all the figures that need be written down in machine computations and shows the number of decimal places that should be retained at each stage of the calculation. The column of products, wx and wy, should first be formed; no rounding-off is recommended here. These figures serve a double purpose; they give by summation the values of $\Sigma\,(wx)$, $\Sigma\,(wy)$ and hence $\bar{x}$ and $\bar{y}$, and they facilitate the calculation of $\Sigma\,(wx^2)$, etc.

(i) $\bar{x}$ and $\bar{y}$ should be calculated to six decimal places. They will be multiplied by about 281 and 323 respectively in finding XY and YY, and the latter are required to be correct to four decimal places.

(ii) $\Sigma\,(wx^2)$ is obtained by taking the sum of products of wx and x. The correction term $\bar{x}\Sigma\,(wx)$ can be subtracted on the machine, since $\bar{x}$ and $\Sigma\,(wx)$ have already been written down.

(iii) These expressions give, by taking the square root, the standard errors of a and b if these are of interest; the expressions are also required later in the calculation.

(iv) The expressions $(\bar{x}_p - \bar{x}_r)$ and $(\bar{y}_r - \bar{y}_p)/b_c$ need not be written down separately for the calculation of M, but the latter is useful for the calculation of s_M.

(v) To calculate s_M without writing down any intermediate figures, first square $\left(\frac{\bar{y}_r - \bar{y}_p}{b_c}\right) = 0{\cdot}04167$. Multiply the answer by $V\,(b_c)$ and add the other two terms under the square root. The square root is then found, put on the machine, and divided by b_c to give s_M. Irwin uses the notation σ_M for s_M. The use of s_M, however, accords with the general convention in statistics that Roman letters refer to estimates and Greek letters to population values.

(vi) The formula given for the standard error of the relative potency (antilog M) is an approximation, which is quite satisfactory when, as in this example, s_M is small compared with M.

Two tests of significance may be wanted: (1) to test whether the potencies of the two poisons which are being compared in a single experiment are significantly different, i.e. whether the relative potency is significantly different from unity; (2) to test the significance of the difference between the relative potencies of two poisons which have been compared, in different experiments, with the same control. Both these tests should be made in the log scale.

To test whether M differs significantly from zero, we may regard s_M

(as derived in the specimen calculation) to be based on (n_1+n_2+1) degrees of freedom, where n_1, n_2 are the numbers of degrees of freedom in the χ^2 tests for homogeneity of the two poisons. In the present example, $n_1=n_2=2$. This test is sufficiently accurate if $t^2 V(b_c)/b_c^2$ is small compared with unity, where t is the value of Student's t, for (n_1+n_2+1) degrees of freedom, at the level of significance desired. In the present example, at the 5 % level, $t=2{\cdot}571$ and $t^2 V(b_c)/b_c^2$ is 0·045.

The exact test of significance will rarely be required, but may be made by regarding the relative potencies as significantly different if

$$\bar{x}_2-\bar{x}_1+\frac{(\bar{y}_1-\bar{y}_2)}{b_c(1-\lambda)}-\frac{t}{b_c(1-\lambda)}\sqrt{\{1/\Sigma(w_1)+1/\Sigma(w_2)\}(1-\lambda)+\frac{(\bar{y}_2-\bar{y}_1)^2 V(b_c)}{b_c^2}}$$

is greater than zero, where $\lambda=t^2 V(b_c)/b_c^2$, the suffices 1 and 2 being chosen so that M is greater than zero.

To test the significance of the difference between the values of M obtained in two different experiments for two poisons which have been compared with the same control, we refer $\dfrac{M_1-M_2}{\sqrt{s_{M_1}^2+s_{M_2}^2}}$ to the t-table, with the sum of the number of degrees of freedom in s_{M_1} and s_{M_2}.[1]

As an example, we may test whether the potency of the toxicarol precursor in series 2, Table I is significantly different from that of the toxicarol precursor (by potash separation) in series 3, both having been compared directly with rotenone.

$$M_1=-1{\cdot}1863,\quad M_2=-1{\cdot}1137,$$

$$s_{M_1}=\ 0{\cdot}0287,\quad s_{M_2}=\ 0{\cdot}0420.$$

Hence $$t=\frac{M_1-M_2}{\sqrt{s_{M_1}^2+s_{M_2}^2}}=\frac{-0{\cdot}0726}{0{\cdot}0509}=-1{\cdot}43,$$

with 8 degrees of freedom, which is not significant.

As a second example, compare the Sumatra-type resin in series 4 with the *D. elliptica* resin in series 6.

$$M_1=-0{\cdot}800,\quad M_2=-0{\cdot}5653,$$

$$s_{M_1}=\ 0{\cdot}0247,\quad s_{M_2}=\ 0{\cdot}03225.$$

Hence $$t=\frac{M_1-M_2}{\sqrt{s_{M_1}^2+s_{M_2}^2}}=\frac{-0{\cdot}235}{0{\cdot}0406}=-5{\cdot}79,$$

[1] This test must, of course, be used with caution. It would scarcely be relevant to compare two poisons from two experiments carried out under widely differing conditions.

with 10 degrees of freedom. This value is highly significant, the 1% point being 3·17. Thus the potency of the *D. elliptica* resin is significantly greater than that of the Sumatra-type resin.

Where the values of s_{M_1} and s_{M_2} differ so widely that it is clear that one of the pair M_1, M_2 is determined much more accurately than the other, no exact test of significance can at present be given. In this case, which does not arise in any of the present experiments, it is safest to give to the value of t the number of degrees of freedom in the *larger* of s_{M_1} and s_{M_2}, instead of the sum of the number of degrees of freedom in s_{M_1} and s_{M_2}.

SUMMARY

1. An account is given of the preparation and a few of the properties of a compound isolated from the extracts of Sumatra-type derris root.

2. This compound although in the present investigation containing extraneous material, probably sumatrol, yields optically inactive toxicarol in high yield, and is characterized by the switch-over from laevo- to dextro-rotation on the addition of caustic potash in methyl alcohol to its benzene solution, and is mainly responsible for this feature of the Sumatra-type resins under similar treatment. The change-over in rotation was followed by a gradual fall in rotation of a unimolecular type. The compound is laevo-rotatory in benzene and dextro-rotatory in alcohol.

3. The toxicities to *Aphis rumicis* of rotenone, toxicarol precursor, sumatrol, toxicarol and the residual resins from the Sumatra-type and *Derris elliptica* roots have been determined. In our experiments the toxicity in descending order was Rotenone > *D. elliptica* resin > Sumatra-type resin > sumatrol = toxicarol precursor > inactive toxicarol.

4. The statistical method used in analysing the insecticidal data is described in an Appendix.

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NOTE

A FURTHER NOTE ON FUNGUS ASSOCIATION IN THE SIRICIDAE

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An investigation into the relationship existing between fungus and insect in the Siricidae was commenced in 1928 on the suggestion of Dr R. Neil Chrystal.

Buchner (1928) had described and figured the occurrence in *Sirex gigas* of paired glandular structures which occur at the base of the ovipositor. These glands open into the insect's vagina and in them fungus "oidia" were observed by him. These "oidia" in some cases possessed clamp connexions and the fungus was, therefore, placed in the Basidiomycetes. A note on this investigation was published by the author (Cartwright, 1929), *S. cyaneus* being the species mainly under investigation. *S. gigas* was only cursorily examined owing to lack of adequate material. Since this note was published the investigation has been continued as material became available and as time permitted. Material of both *S. cyaneus* and *S. gigas* has been received from several different localities, and a limited amount of material of *Xiphydria prolongata*. It is proposed, when the entomological side of the investigation has been completed, to publish a full account, but a summary of progress is given here in the hope that it may prove of interest to other workers in the same field.

Since the publication of the original note (Cartwright, 1929) the results there recorded in respect of *Sirex cyaneus* have been confirmed. A fungus has been isolated from glands, eggs, oviposition and larval tunnels of both *S. cyaneus* and *S. gigas*. It has not been possible to establish with certainty the identity of the fungus associated with *S. cyaneus*, but it closely resembles that obtained from *S. gigas* and, although there are certain constant differences, these are hardly sufficient to warrant considering it more than a form of the same species. The culture is characterized by the formation as it ages of a greyish lilac colour, this coloured area possibly representing a rudimentary hymenial surface. The colour resembles that of the fruit body of *Peniophora quercina*.

The mycelial characters of the fungus associated with *Sirex cyaneus* are very similar to those found in *Stereum sanguinolentum*, and cystidia with crystalline incrustations are produced, but in greater abundance. In particular, the sweet odour characteristic of *S. sanguinolentum* is pronounced. A few basidiospores were produced in one culture and these came within the range of measurement of those of *S. sanguinolentum*. The rate and type of growth on malt agar is also very similar. On the other hand, a constant difference is apparent between typical *S. sanguinolentum* and all isolations from *Sirex cyaneus*. This is the pinkish cinnamon colour produced in cultures of the latter as compared with the more yellowish tints produced in the former. Another point of difference is that the fungus in the case of *S. cyaneus* appears to develop a definite mycelial growth which invests the egg previous to oviposition, whereas in the case of *S. gigas* the short segments remain in this condition until after

oviposition, but an insufficient number of insects have been so far examined to state whether this difference is a constant one. As there is a fairly wide variation in cultural characteristics between different isolations of *Stereum sanguinolentum*, it is thought probable that the fungus associated with *Sirex cyaneus* is a form of *Stereum sanguinolentum*, although, until characteristic fruit bodies are obtained, it is not possible to confirm this opinion.

The fungus isolated from *Sirex gigas* has been identified by means of comparison with standard cultures as *Stereum sanguinolentum*. Small samples of larch were inoculated with a culture isolated from a gland of *Sirex gigas*, and fruit bodies of *Stereum sanguinolentum* were produced on the wood, thus confirming the identification. Further corroborative evidence was obtained by the formation of fruit bodies of this fungus appearing on wood samples from which *Sirex gigas* had emerged after these had been kept moistened in a potato dish in the laboratory.

Under the moisture conditions occurring in wood in which live larvae are present no advanced stage of rot has been observed although, in a specimen of larch wood taken from a felled log in which old *Sirex* attack was evident, the decay had progressed to a considerable extent. In one instance a pupa of *S. gigas* was sent to me by Dr Chrystal, which was dead and surrounded by a weft of mycelium. The fungus proved to be *Stereum sanguinolentum*. In this case the moisture conditions had apparently become favourable for the rapid development of the fungus, with fatal results to the *Sirex*. It appears that conditions of moisture in the wood favourable to *Sirex* are those that are just sufficient to support a slow development of the fungus.

In both the case of *S. cyaneus* and *S. gigas* there is very strong evidence for stating that a definite species of fungus is associated with each species of insect in so far as this country is concerned, and further evidence to the same effect has been obtained by Clark (1933) working in New Zealand with *S. noctilio*.

Females of both *S. cyaneus* and *S. gigas* have been observed ovipositing in wood free from all traces of fungus; the tunnels have been opened up and the eggs removed immediately after oviposition, and also at varying times after oviposition. Sections cut across these oviposition tunnels show all stages of development of the fungus from the egg, so that no doubt remains but that the fungus is introduced into the wood during oviposition.

Clark (1933) in New Zealand has carried the work a stage further, claiming to have found fungus in the larva apart from that which is obviously present in the digestive system owing to the larva feeding on wood containing fungus. In my preliminary examination, fungus was observed in a partially digested condition in the larval gut. Sections across late stage pupae also showed fungus to be present in glands at the base of the ovipositor in the case of female pupae.

Work has now commenced following up the development of the special fungus-carrying glands with a view to finding at what stage in the life history of the insect the fungus becomes segregated in these structures; this is being investigated by my entomological colleagues.

Xiphydria prolongata.

A small quantity of willow containing this insect has also been examined. Up to the present the investigation has been confined to the isolation of the fungus from the adult female and from the egg and larval tunnels. From the original material

received, *Stereum purpureum* was isolated from old larval tunnels, but from material in which larvae were still active, and from which adults emerged later, isolations both from larval tunnels and from glands from the adult female have given a culture resembling closely in macroscopic appearance that of *Daldinia concentrica*, but differing from it microscopically in certain respects.

The fungus belongs almost certainly to the Pyrenomycetes and is one which can cause some decay of the wood.

Summary

1. Earlier work on the association of *Sirex cyaneus* with a Basidiomycete fungus has been confirmed. The culture of the fungus closely resembles, but is not identical with that of *Stereum sanguinolentum*; it is therefore thought that it is a form of *S. sanguinolentum*.

2. The fungus isolated from *Sirex gigas* has been identified as *Stereum sanguinolentum*.

3. At the moisture content favourable for the development of the larvae, only slow development of the fungus can take place. Under more moist conditions vigorous fungus growth occurs, with apparently fatal results to the larvae.

4. The fungus is introduced into the wood during oviposition in the case of both *Sirex cyaneus* and *S. gigas*.

5. Fungus was found to be present in glands at the base of the ovipositor in late stage female pupae.

6. A fungus, the culture of which resembles in appearance that of *Daldinia concentrica*, has been isolated from larval tunnels and from glands in adult females of *Xiphydia prolongata*.

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REVIEWS

Statistical Methods. By G. W. Snedecor. Pp. xiii + 341. Ames, Iowa Collegiate Press Inc. 1937. $3.75.

No statistician whose occupation brings him into contact with biological research workers can be ignorant of the urgent demand to-day for a book which will explain statistical methods clearly and authoritatively. It is such a book that Prof. Snedecor has set out to write. In this respect the book perhaps calls for two reviewers, for while the professional statistician may judge with confidence the correctness and utility of the methods explained, he is naturally somewhat handicapped in estimating the appeal which the book will have to a biological research worker with no previous knowledge of statistics.

The author of an introductory text-book must choose whether to present the subjects discussed in a logical sequence or in order of increasing difficulty. The first alternative makes the book more difficult to read, but may result in a more complete and satisfying picture of the whole subject, once the difficulties have been mastered. Snedecor's experience in teaching statistics has led him to choose the latter method. This method certainly makes every individual chapter in the book easy to follow, but its success in presenting the whole subject depends on the ability of the reader to collate discussions widely separated in print. In this task he is, however, given every assistance by the author.

The book opens with a discussion of the test of significance of the departure of an observed percentage from a hypothetical value (the χ^2 test for a single degree of freedom). Then follows an account of a replicated experiment with two treatments only, in which the meaning and calculation of the standard error are explained. Chapters III and IV are devoted to sampling from a normal population, and introduce the reader to the t-test and to the idea of fiducial probability. Chapter V gives some useful short cuts in computation. The succeeding chapters discuss in turn linear regression, correlation, large sample theory (including the use of grouping and the tests of departure from normality) and we return in Chapter IX to the χ^2 test, with more than one degree of freedom. The chapter on correlation is excellent; it stresses the limitations of the correlation coefficient both in its utility and in its ability to measure association.

Thus far, the succession of subjects is logically somewhat haphazard. The remainder of the book, with the exception of the last chapter, concerns the analysis of variance, and includes the analysis of co-variance, multiple regression, the fitting of curved regression lines and the isolation of single degrees of freedom. The final chapter discusses briefly the binomial and Poisson series.

Prof. Snedecor's style is deliberately conversational, and while the purist may deplore the large number of colloquialisms, the friendly atmosphere which is created is a great help in studying a subject which is often approached by the student with a mixture of awe and hostility. Examples to be worked by the reader are numerous in all parts of the work. In some cases, working of the examples is not merely helpful but essential to obtain a correct understanding of the discussion; e.g. on p. 237 the treatment given in the text is superficial and is a bad precedent for the reader to follow; this is, however, put right in example 12.10, where the general conclusion reached in the text is corrected.

The methods developed are in the main sound and practical. A few points occurred to the reviewer which might mislead the reader. On p. 178, the statement occurs: "The F-test is valid whether the variance is homogeneous or not." Presumably the author means the F-test considered as a general test of differences between samples; however the F-test is always used in practice to discriminate

between *means* and as such is not a correct test if the variances differ widely. On p. 196 a large sample test of the differences between a set of variances is used on a set of variances with only three degrees of freedom each, to which it is certainly not applicable, though the author warns the reader elsewhere of the limitations of large sample methods. The treatment of multiple regression might be more simple and self-contained if the use of the multiple correlation coefficient were omitted. The book ends on an unnecessarily defeatist note, the last sentences reading: "If large samples of enumeration data do not follow either Binomial or Poisson distributions, there is no alternative but the disagreeable one of treating them as normal. Conclusions, if any, should be tentative." Thanks to the use of transformations, this statement is fortunately untrue, as a recent paper in this *Journal* exemplifies (C. B. Williams (1936), **24**, 404). Indeed, the neglect of transformations, in particular the square root, inverse sine and log transformations, is a serious limitation to the use of this book.

Prof. Snedecor is to be congratulated on producing a book which will be of great assistance to biological workers.

W. G. COCHRAN.

Report of the Fourth International Grassland Congress. Edited by R. O. WHYTE. Pp. xxxiv + 486. Obtainable from the Joint Secretaries, IVth International Grassland Congress, Aberystwyth. 1937. 40*s*.

This *Report* contains lists of the officers of the Congress, a table of contents, full texts of the Presidential Address, plenary papers, sectional papers, and discussions, the names and addresses of persons attending the Congress, and indexes of authors of papers and contributors to discussions.

A Grassland Congress held within the British Empire could have but one President, R. G. Stapledon, a man of vision and courage whose leadership is everywhere recognized. But in an opening address to a large Congress, any President is almost compelled to range widely and superficially and so, although what Stapledon said is well said and well worth saying, it could hardly be expected to contain anything new.

The authors of the thirteen plenary papers represent nine countries; mostly deal with somewhat wide topics of special importance in their particular countries; and give a good idea of present viewpoints. Grass drying, which is much to the fore in Great Britain, is discussed by Woodman of Cambridge. Vezzani and Carbone of Torino deal with alpine grazing of cattle, a primary issue in Northern Italy. More than 70% of the occupied land of Australia south of the tropic of Capricorn is used for wool growing and Marston of Adelaide, therefore, discusses the nutritive value of pastures for wool production. American plant breeders are perfecting techniques of grass breeding, and clarifying objectives so as properly to relate plant breeding to other grassland improvement activities. Cardon of Washington deals, therefore, with plant breeding in relation to pasture improvement. Sweden is the home of the ecotype concept, and Sylvén of Svalöf considers the importance of ecotype formation for the breeding of herbage plants. In Sweden, grasslands occupy nearly one-half of the total agricultural area and, as grassland management is in a transition state, Osvald of Uppsala discusses achievements and aims in modern Swedish grassland management. At Aberystwyth one of the main themes has been strain building in the herbage grasses and this subject is dealt with by Jenkin. Pedigree grasses need to be multiplied, distributed and grown, and the part played by seedsman and farmer is considered by Miln of Warrington. In New Zealand, about 14 million acres of forest have been felled and sown to grass, and about 2 million acres of fern and scrub land: awaiting development are still about 2 million acres of fern and scrub land and some 4 million acres of standing forest. This problem of the conversion of rain forest to grassland is discussed by Bruce Levy of Palmerston North. It has long been known that non-legumes derive benefit when grown in association with legumes, but the phenomenon has presented obscurities. During the last decade it has been studied by Virtanen of Helsinki who puts forward an explanation. In Canada the few species of grasses and legumes that are grown extensively are highly adapted to the rather exacting climatic

conditions, and Kirk of Ottawa discusses their evaluation for pasture. In world agriculture soil erosion has become an imperative issue, and a recent survey has shown that, in the United States, about 100 million acres are so seriously eroded as to be practically worthless for continued cropping, while a further 100 million acres are rapidly becoming worthless. The outstanding value of pastures in soil erosion control is now recognized and is discussed by Enlow of Washington. In Germany, grassland research is well advanced, and enables Klapp of Bonn to consider the principles governing the value of herbage plants for hay and pasture use. These plenary papers contain nothing strikingly new, but they are interesting and serve to emphasize some of the main general issues confronting world agriculture to-day.

The Congress was organized in six sections as follows: (1) Grassland ecology, including range management; (2) Seeds mixtures, legumes for use in poor pastures; (3) Plant breeding, genetics and seed production; (4) Manures and fertilizers, soil aspects of grassland; (5) Nutritive value of pastures, fodder conservation; (6) Pastures. Management, yields and economics. To a large extent the sectional papers deal with the same general problems as the plenary papers but help to fill in the details and, as the contributors represent eighteen countries, the papers embody an unusual width of knowledge and experience. It is not possible to review the fifty-five sectional papers, or even to mention individual contributions. On the whole the papers are of high quality and many of them are exceedingly interesting. Grassland diseases are not considered, and only one paper is devoted to grassland pests.

During an evening spent in browsing these pages two general ideas gradually formed in my mind. The first was that in spite of the enormous and rather diffuse range of grassland problems, with their apparent complexities, and their manifold impacts upon all sorts of correlative agricultural, economic and social issues, the general principles underlying the utilization and development of grasslands are few in number and comparatively simple. The second was the fundamental importance of grasslands in social economy and, arising out of this, the certainty that the improvements which could be effected in grasslands within a relatively short period would so materially influence the economic and social structure of many countries, that the changes would amount to a sizable revolutionary movement. Friend Stapledon will have much to answer for.

The *Report* is a good cross-section of a vital subject and a valuable storehouse of data and viewpoints. The Editor has had no light task, and must earn praise for the way in which the volume has been produced and for its rapid issue after the Congress. The *Report* would have been graced by a good portrait of the rather elusive Congress President.

WILLIAM B. BRIERLEY.

Studies on Wheat Grown under Constant Conditions: a Monograph on Growth. By H. L. VAN DE SANDE-BAKHUYZEN. Pp. xvi+400. Food Research Institute, Stanford University, California. 1937. $4.00.

In biology there are, as yet, no exact values which can be used as the hydrogen atom is used as a basic datum in chemistry, or the 100% in mathematics. If there were a standard plant with which any other plant could be compared, botany would immediately become a much more exact science. The rapidly accumulating knowledge of the nutritional requirements of plants and the development of the technique of growing plants in chambers in which environmental conditions are strictly controlled, give promise that it might be possible to obtain such an intensive knowledge of a single plant that that plant could serve as a datum line in botany. A selected pure line of wheat grown under constant conditions might well be accepted as such a standard plant, and the importance of the present work lies in the fact that it may be regarded as a first approach to such an ideal. The book has grown out of crop forecasting studies and the fact that some of the correlations observed could not be explained on the basis of existing knowledge regarding the physiology of growth.

It was decided, therefore, to make a study of the wheat plant organ by organ under an unvarying environment, and to use this knowledge as a standard with which to compare the growth with single variables modified.

Chapter I is an admirable general introduction to the physiology and development of the wheat plant. Seven chapters follow dealing with the growth curve in annual plants, six with materials and methods, three with general growth features, two with dry weight and moisture content of the different organs, and four with dry weight and moisture of the "standard plant". The next two chapters discuss and summarize these data. There follow four chapters dealing with the nitrogen and carbon of the organs, six with nitrogen metabolism in relation to growth and development, and a final chapter in which these data are discussed and summarized. The book concludes with a useful bibliography citing references up to 1935 when the manuscript was completed in almost its present form, and an index. Much of the first nineteen chapters is reprinted with modifications from *Physiological Reviews* and *Plant Physiology*; and Elizabeth P. Griffing and Carl L. Alsberg have contributed to the sections dealing with the carbon and nitrogen content of the wheat plant.

Quite apart from the intrinsic value of the data, the work is of importance in that it lies within a comparatively untouched field. There have, of course, been numerous researches on the pathological relationships of plants growing under controlled conditions, and on various aspects of the physiology of detached organs maintained under controlled conditions, but there has been astonishingly little investigation of the general physiology of complete plants growing in a determined environment. In the present work the factors controlled were temperature, light, humidity of the air, moisture and salt content of the substratum (sand or solution). The present work is to be regarded rather as the initiation of a programme for future work than as the completion or solution of a problem. Even so, however, it is of the greatest interest and its continuance may easily lead to a new valuation of some of the more fundamental concepts and problems of plant physiology. By itself the attempt to work out the idea of a "Standard Plant" well justifies publication.

WILLIAM B. BRIERLEY.

Phytohormones. By F. W. WENT and K. V. THIMANN. Pp. xi + 294. New York: The Macmillan Co. 1937. 17*s*.

The birth of the growth hormone concept *per se* may be dated 1919 with the publication of Paál's second paper on the phototropic stimulus. The suggestion of the existence of organ-forming and correlative substances goes back to the writings of Duhamel du Monceau in 1758 and was re-stated in different terms by Sachs (1880–93) and by Beijerinck in 1897, while Darwin conceived the idea of phototropic and geotropic curvatures being due to "some influence" which "is transmitted from the upper to the lower part, causing the latter to bend". Rothert, Fitting, and Boysen-Jensen laid more of the experimental foundation, but it was left to Paál to show the relation of the phototropic response to a material substance which is active in promoting *normal* growth.

The formulation of a clear-cut concept gave a great impetus to experimental study and the final material proof was provided by the skilful chemical work of Kögl, Haagen Smit, and Erxleben in isolating and analysing the auxins and in showing that these substances produced reactions identical with those previously obtained with root and shoot tips.

The establishment of the relation of these growth-promoting substances to many well-known correlations in organ-development, such as bud inhibition and root initiation, has led not only to the elucidation of many previously obscure problems but to the development of an entirely new outlook on the physiology of growth and behaviour in plants. It is, possibly, not too much to suggest that the hormone concept may rank in the history of plant physiology with the cell-theory in that of morphology or the binomial principle in systematics. As the authors themselves say, "we already

see the auxins and their properties as a continuous thread connecting most of the developmental and growth processes in the plant".

Indicative of the vast amount of work which the concept has stimulated is the fact that although the theory is not yet out of its teens two important books, both containing solidly condensed experimental results, should already have appeared, the present volume and that of Boysen-Jensen (*Die Wuchsstofftheorie*). Of the two, there can be no question that the book under review is far the more convincing, comprehensive and stimulating. It is only to be expected that two investigators who have themselves contributed a large part of the soundest fundamental work but who have, at the same time, an imaginative and synthetic outlook should occasionally tend to explain too much from too little. We cannot, as yet, understand certain of the phenomena associated with the action of growth-substances, nevertheless, an hypothesis is suggested in almost every case. It is, however, just in this respect that the book is so stimulating.

The main point on which issue will be joined by many physiologists is the tendency to discount the nutritional outlook and to explain, not only to relate, all growth phenomena in terms of auxin production and distribution. For example (p. 231), "the root system probably forms a factor or factors necessary for shoot growth and *hence* the increased root system increases the shoot" (italics are ours), or again, and even more categorically, (p. 232) "quantitative relations between different parts of the plant are expressions of the quantitative relation between auxin and its growth effects. This generalization can now supersede the older view that such growth relations are determined by the amounts of food material present". Truly a revolution in outlook.

Those who seek in this book for a lengthy and detailed treatment of the practical applications of growth substances in horticulture, especially in the rooting of cuttings, will be disappointed. The latter subject receives a bare two pages of text and one illustration, and the very considerable number of papers on it are scarcely noted, four references only being quoted. It is to be hoped that in future editions this section will be expanded, even though the book is primarily a discussion of the fundamental principles of the subject.

There can be no doubt that the work represents an immensely important contribution to plant physiology and it is to be hoped that it will be read and deeply considered not only by the believers but, also, by the diehard minority who still dismiss the hormone concept as a will-o'-the-wisp.

The book is divided into fourteen chapters. After a brief review of the development of the concept, a condensed but adequate account is given of the technique of auxin determinations, followed by chapters on the formation, distribution and chemistry of the auxins. Theories of the mechanism of action are discussed at length, the remaining chapters being concerned with the tropistic and correlative activities of the substances, and general conclusions and future outlook. The book concludes with a bibliography of 569 references, further evidence of the intensive study of the most productive physiological conception of this century.

R. H. STOUGHTON.

A Review of the Literature on Stock-Scion Incompatibility in Fruit Trees, with Particular Reference to Pome and Stone Fruits. By G. K. Argles. Pp. 115. Tech. Communication No. 9. Imperial Bureau of Fruit Production, East Malling, Kent. 1937. 5*s*.

A good practical review of this problem under the general headings: Discussion on the manifestations or symptoms of incompatibility; Possible causes of incompatibility; Stock-scion incompatibility in relation to individual species and varieties of deciduous fruit trees; Conclusions and suggestions. The above occupies the first half of the bulletin and is followed by two pages containing interesting suggestions for further research under the headings: Factors governing the success or failure of

buds and grafts to form a union; The growth of trees on different rootstocks; Tests to determine the mechanical strength of unions; The anatomical structure of unions; Translocation in grafted trees; Physiological and biochemical studies. There is a bibliography of 194 references. A 46-page appendix contains six lists of rootstocks which have been used, either experimentally or commercially, for varieties of pears, plums, peaches, apricots, almonds, and cherries. Apples are omitted owing to the relatively few cases of incompatibility which have been reported. The lists are arranged in parallel column under species, variety and method of propagation of the stock, species and variety of the scion, behaviour of the union, where the observations were made, and reference citation.

WILLIAM B. BRIERLEY.

Forest Bibliography to 31st December, 1933. Compiled and published by The Department of Forestry, University of Oxford. Part 1, pp. xviii + 1–78. 1936. 5*s*. Part 2, pp. 79–199. 1937. 12*s*. 6*d*.

A systematic referencing of current forest literature was begun at the Oxford School of Forestry in 1920 and, after 1924, continued jointly by the School of Forestry and the Imperial Forestry Institute, the bulk of the work being done by Mr P. S. Spokes. At first publication was not intended, the object being to keep staff and students in touch with the latest literature bearing on forestry, but the index was found so useful that, in response to requests from various sources, it was decided to publish the work. The Bibliography comprises literature published to the end of 1933 and contained in the library of the Department. English publications are covered fairly completely and a considerable amount of French and German literature is also included, but few publications in other languages are represented unless they contain English, French or German summaries.

Part 1 commences with 14 pages of the names of journals, and of series of bulletins, reports, etc., with their abbreviated titles. Many of these are of very special character and so omitted from the Oxford *World List of Scientific Periodicals*, but it is a great pity that the standard abbreviations of such journals as are included in this work could not have been adopted. Four pages are then devoted to a Subject Classification which forms the basis of the bibliographical arrangement. The major headings are: A, General Forestry; B, Silviculture; C, Forest Protection; D, Forest Utilization; E, Forest Mensuration; F, Forest Valuation and Finance; G, Forest Management; H, Forest Policy and Economics; I, Meteorology; J, Geology and Soil; K, Education and Research; L, Terminology and Classification of Information; M, Engineering and Surveying; N, Botany; O, Invertebrate Zoology; P, Bibliography. Each section is further subdivided under headings. The two parts of the Bibliography now issued cover the literature to the end of Silviculture (i.e. A and B). The citations are grouped under two heads: (1) periodicals (2) other publications; books are excluded. The detailed arrangement on the pages is by parallel columns under date: year, abbreviated title of journal, volume, page, title of paper, and name of author.

When it is realized that about 750 journals, etc., are dealt with, some dating as far back as the early nineteenth century, the magnitude of the task may be appreciated. The indexing has been done with meticulous care and, when completed, the Bibliography will be an indispensable work of reference.

WILLIAM B. BRIERLEY.

Applied Mycology and Bacteriology. By L. D. GALLOWAY and R. BURGESS. Pp. ix + 186. London: Leonard Hill, Ltd. 1937. 10*s*.

Applied microbiology is a large and rapidly developing field of research, and whilst numerous volumes dealing with special aspects and problems are available, there has been no up-to-date book of convenient size in which the whole field was surveyed.

The present book fills the gap admirably since both authors have had wide practical experience of the subject.

The book is divided into two parts. Part 1, with eight chapters, is in a sense introductory, and deals with the generalities and classification of the fungi and bacteria, methods for their study, their metabolism, and their control. The material is well chosen and includes the essentials, and the authors have condensed readably into small compass a mass of data and sound practical knowledge. Part 2, with six chapters, is devoted to the applications of microbiology in the food, fermentation and textile industries, medicine and hygiene, agriculture, and various miscellaneous problems. This portion occupies only 73 pages, but the scope of the treatment and the amount of matter contained are astonishing. The authors state that "Medical microbiology has been barely touched on, and admittedly deserves a larger space than it receives here." On the other hand this is one of the specialized applications on which there is a superabundance of readily available books, and the authors have been wise in restricting their attention to the more general hygienic issues. The final miscellaneous chapter deals briefly with timber decay, wood pulp and paper, rubber, leather, paints, moulds and arsenic compounds, tea, coffee, cocoa, indigo, tobacco, vegetable oils, the use of micro-organisms for chemical analysis, and a final interesting section on future developments in economic microbiology. Key references are cited at the end of each chapter, and these are commendably up to date. The book closes with an adequate index.

This book is the best introductory treatment of the subject I know. It would form an excellent basis for a lecture course on applied microbiology, or first-class reading for any student of botany who might wish to adventure beyond the academic pale of his mycological studies.

WILLIAM B. BRIERLEY.

A List of Missouri Fungi. By W. E. MANEVAL. Pp. 150. University of Missouri Studies. 1937. xii (3). $1.25.

A list of plant pathogens and wood-destroying fungi including, also, a small number of saprophytes, bacteria, nematodes, and diseases caused by viruses and environmental conditions. The organisms are arranged alphabetically by genus and species, with host plants listed under each species and, in all, there are some 1330 organisms and diseases on about 700 different hosts. There is a host index with the fungi, etc., arranged alphabetically under each host species. The volume opens with a review of the literature dealing with Missouri fungi and closes with a bibliography of 526 references. The work seems to have been well done and there are commendably few misprints.

WILLIAM B. BRIERLEY.

Technique of Grass Seed Production at the Welsh Plant Breeding Station. By GWILYM EVANS. Herbage Publication Series, *Bull.* No. 22. Pp. 36, 24 figures. Aberystwyth: Imperial Bureau of Plant Genetics. 1937. 5*s*.

The grasses bred at the Station are *Lolium perenne, L. italicum, Dactylis glomerata, Phleum pratense, Festuca pratensis, F. rubra, F. ovina, F. arundinacea, Alopecurus pratensis, Poa pratensis, Holcus lanatus, Anthoxanthum odoratum, Phalaris arundinacea* and *P. tuberosa.* The first stage multiplication from a limited number of finally selected plants is carried out at or near the Station; the second stage multiplication on a limited field scale, and the third stage with several fields for each strain, by growers in favourable districts. The three stages are described, the greater part of the bulletin concerning stage three, where details are given regarding selection of centres, field management, harvesting, treatment of harvested crops, successive seed crops,

and crops to follow grass seed crops. There are notes on the production of seed for general distribution and on the bred strains of grasses and clovers released by the Station.

WILLIAM B. BRIERLEY.

Production of Grass Seed. Herbage Publication Series. *Bull.* No. 19. Pp. 46. Aberystwyth: Imperial Bureau of Plant Genetics. 1937. 5*s*.

An international exchange of opinions and experiences on the technique of producing seed of *Alopecurus pratensis*, *Bromus inermis*, *Cynosurus cristatus*, *Dactylis glomerata*, *Phleum pratense*, *Poa pratensis*, and various species of *Agropyron*, *Agrostis*, *Festuca* and *Lolium*. Contributors represent Scotland, Northern Ireland, Canada, New Zealand, Germany, Sweden, and U.S.A.

WILLIAM B. BRIERLEY.

Collection of Native Grass Seed in the Great Plains, U.S.A. By F. J. Crider and M. M. Hoover. Herbage Publication Series, *Bull.* No. 24. Pp. 8, 12 figures. Aberystwyth: Imperial Bureau of Plant Genetics. 1937. 2*s*.

The grasses included are *Agropyron smithii*, *Andropogon scoparius* and *A. furcatus*, *Buchloë dactyloides*, *Sporobolus airoides*, and *Bouteloua gracilis*.

WILLIAM B. BRIERLEY.

Production of Legume Seed. Herbage Publication Series, *Bull.* No. 23. Pp. 48. Aberystwyth: Imperial Bureau of Plant Genetics. 1937. 5*s*.

An international exchange of opinions and experiences on the technique of producing seed of *Anthyllis vulneraria*, and various species of *Glycine*, *Lespedeza*, *Lupinus*, *Medicago*, *Melilotus*, *Trifolium*, and *Vicia*. Contributors represent Australia, Canada, Great Britain, India, Czechoslovakia, France, Germany, Hungary, Sweden, and U.S.A.

WILLIAM B. BRIERLEY.

Weeds, Weeds, Weeds. By Sir Charles Vernon Boys. Pp. 69. London: Wightman and Co., Ltd. 1937. 1*s*.

A pleasantly written and popular little book describing the author's personal experiences in the control of weeds by various chemicals, and containing much sound advice. The author shows unusual abandon in his use of small and capital letters in the spelling of scientific names.

WILLIAM B. BRIERLEY.

Recent Advances in Entomology. By A. D. Imms. London: J. and A. Churchill, Ltd. 2nd edition. 1937. 15*s*.

Dr Imms has once more increased our indebtedness to him by the publication of the second edition of *Recent Advances in Entomology*. The subjects dealt with are the same as in the first edition (noticed in this *Journal* in 1931, vol. **18**, p. 262) but the book has been enlarged by some 55 pages and many of the chapters have been largely re-written. The field of work open to entomologists is now so vast that none

of us can hope to keep abreast of all advances in knowledge. Dr Imms gives us, as we have learned from his *Text-book* and his first edition of *Recent Advances*, the results of wide and critical reading in what we may call the grammar of entomology on which he is the acknowledged leader in this country.

The first eight chapters of his book are devoted to this and in them his active and critical interest is manifest. In the later chapters on ecology and its applications and on parasitism, too, he sums up skilfully the important new contributions and their implications.

When he deals with the control of insect pests and weeds by the use of parasites, however, his critical lance seems to lie in rest and his account of "biological control" is virtually a repetition of the published views of the authors cited.

Dr Imms follows the majority of workers on the control of insect pests by parasites in using the term "biological control" to mean solely control by parasites. For example, he deals with Parnell's production of the jassid resistant cotton U4 in his sub-chapter on "resistant varieties" although surely Parnell's work is one of the finest examples of the biological control of an insect pest we have had for many years. In the writer's view, this restriction of the term biological control is much to be regretted, both on theoretical and practical grounds. Dr Imms, however, apparently agrees with the restriction for he states the "entomological viewpoint" as follows:

"Among the natural agencies exercising a controlling influence upon insect life the complex of meteorological factors constituting climate is ever present. Simultaneously another complex of a biological nature is exerting its influence, and represents the sum total of the activities of bacterial, fungal and other diseases, of insectivorous birds and mammals and of parasites and predators. The restraint exercised by these several biological agencies is known as biological control, and in so far as insect life is concerned, the influence of parasites and predators alone is of immense significance."

It is striking that, in enumerating the agencies which govern fluctuations in insect numbers, Dr Imms completely omits the plants which afford sustenance for many insects, especially nearly all insects injurious to agriculture and forestry. It is the more remarkable because in the sentence which immediately follows the quotation above, he says: "Plant life is likewise affected by a complex of biological agencies including fungal, bacterial, and other diseases, together with the depredations of those *insects directly dependent upon vegetation for their sustenance*."[1]

Why is it that in nearly all discussions on the fluctuations of insect populations conducted by those interested in the parasitic hymenoptera and diptera the sustenance factor is ignored? One would almost think that Malthus, who by his axiom that all animal life tends to increase to the maximum of its subsistence, inspired both Darwin and Wallace to consider the origin of species by natural selection, is completely forgotten. In spite of his last statement quoted, Dr Imms proceeds immediately to discuss "Biological Control of Insect Pests" and once more reverts to a discussion in which the plant is forgotten and insect pest populations are isolated from their environment except in so far as parasites are concerned. In fact a few lines later Dr Imms refers to Dr Nicholson's papers on the principles underlying control by parasites and particularly emphasizes Nicholson's reference to the "fact that if there is a balance in the population of a particular insect some of the factors causing mortality must be dependent upon past density and destroy a greater proportion of the individuals of a specific pest when the density of the latter is high than when it is low". He adds that "in the case of parasites and predators it is assumed that they search for their hosts at random, and it is shown that the mortality which they cause will be dependent upon host-density. Since climate and *other factors* operate irrespective of such density, parasites and predators are regarded as the main factors bringing about balance".

Like Dr Nicholson, Dr Imms is prepared to discuss the parasite and its host, the pest insect, and ignore the fact that it also has a host and that the density of the pest host may be dependent on the plant host and that that plant host cannot be

[1] My italics.

ignored. That it should be ignored is the more surprising if we remember that it is the plant host and its protection that is the reason for the whole discussion.

It is entirely desirable that theories of insect populations should be formulated and discussed, but theories must start with premises, and their value will depend almost entirely on whether the premises are sound or faulty. That authors of papers published in scientific journals should sometimes let their zeal for theorizing outrun their common sense and discretion is pardonable; the danger is lest students of Dr Imms' book, still finding their way in entomology, should be misled by the discussion just quoted. Already, at least two schools of thought concerning the factors governing fluctuations in insect populations have appeared among us, the one represented by Prof. Buxton and Dr Uvarov, for example, emphasizing the importance of the climatic factor, the other represented by the late Dr Tillyard and Dr W. R. Thompson, for example, emphasizing the importance of the parasite factor. Both schools are partly right but neither is completely right and the real need is to suspend judgement, to discriminate and above all to acquire more knowledge without bias.

One sympathises with Dr Imms if, in a later edition, he seeks to give a balanced account of insect population problems, because so much is written on parasitism and on climatic factors and so little on the food supply or subsistence factor, but it is much to be hoped that he will make the attempt, for his *Recent Advances*, in other respects, sets a standard of critical compilation which is admired the world over.

J. W. MUNRO.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1937

THE officers and Council of the Association were as follows:

President: J. HENDERSON SMITH, M.B., Ch.B.

Vice-Presidents: S. P. WILTSHIRE, M.A., D.Sc.
C. T. GIMINGHAM, B.Sc., F.I.C.

Hon. Treasurer: J. HENDERSON SMITH, M.B., Ch.B.

Hon. Secretaries: General and Botanical, W. P. K. FINDLAY, M.Sc.
Zoological, G. FOX-WILSON.

Hon. Editors of *Annals of Applied Biology*:
General and Botanical, Prof. W. B. BRIERLEY, D.Sc.
Zoological, C. T. GIMINGHAM, B.Sc., F.I.C.

Council: H. F. BARNES, M.A., Ph.D., A. S. BUCKHURST, A.R.C.S., W. J. DOWSON, M.A., D.Sc., T. GOODEY, D.Sc., H. MARTIN, D.Sc., H. W. MILES, D.Sc., Ph.D., H. C. F. NEWTON, B.Sc., G. SAMUEL, M.Sc., E. R. SPEYER, M.A., H. G. THORNTON, D.Sc., S. P. WILTSHIRE, M.A., D.Sc., H. WORMALD, D.Sc.

Mr H. C. F. Newton was co-opted by the Council to fill the vacancy resulting from the death of Dr Maldwyn-Davies.

The Association has met on six occasions during the year, including one Field Meeting and one afternoon visit. The Annual Summer Meeting was held on 4 June at the Experimental and Research Station, Cheshunt, by kind permission of the Director, Dr Bewley. The afternoon visit on 11 December was to the gardens of the Zoological Society of London. To both these Institutions the Association is indebted for their hospitality.

At ordinary meetings the attendance as recorded in the signature book was, on the average, 33 members and 17 visitors.

Eighteen Ordinary Members were elected during the year and ten Members resigned. The Council has to record with regret the death of four Members, Prof. Borthwick, Dr Maldwyn-Davies, Miss Hoggan, and of Prof. Paine who acted as General and Botanical Secretary of the Association from 1923 to 1926.

The Association now numbers 314 Members including 12 Honorary Members: of the Ordinary Members as far as is known 252 are resident in the British Isles and 50 in the Empire or Foreign Countries.

The following papers and discussions were brought before the Association during 1937:

12 *Feb.* *Recent Work on the Death-Watch Beetle,* Xestobium rufovillosum.
R. C. FISHER, B.Sc., Ph.D.: "Life history studies."
F. R. CANN, D.I.C.: "Occurrence in buildings and methods of control."
Some Recent Developments in Fumigation.
A. B. P. PAGE, Ph.D.: "Applications and distributions of fumigants."
O. F. LUBATTI, Ph.D.: "Detection and determination of fumigants."
The Use of Liquid Insecticides against Warehouse Pests. C. POTTER, Ph.D.

12 *Mar.* *The Independence and Interdependence of Branches.* W. A. Roach, D.Sc.
Notes on Plant Disease Control in the U.S.A. and Canada. R. W. Marsh, M.A.

8 *Oct.* *The Wireworm Problem, with special reference to the North West of England.* H. W. Miles, D.Sc.
The Rook in the Rural Economy of the Midlands. A. Roebuck, N.D.A.
The Food Habits of the Little Owl. Miss A. Hibbert-Ware, M.B.O.U.

5 *Nov.* *The Marssonina Disease of Lettuce.* Miss G. Stevenson.
The Significance of the Climax for British Forestry. A. S. Watt, B.A., Ph.D.

During the past year the Association has again enjoyed the privilege of holding its meetings in the Botanical Department of the Imperial College of Science and Technology and in the Metallurgical Lecture Theatre of the Royal School of Mines, and the Council takes this opportunity of recording its grateful thanks on behalf of the Association to the College Authorities for their valued hospitality.

The Hon. Editors of the *Annals of Applied Biology* report that in 1937 the volume comprised pp. xiv + 940 and 51 plates, as against pp. xiv + 921 and 39 plates for 1936. Including papers published as "Proceedings", vol. xxiv contained 67 papers and 44 reviews, as against 64 papers and 32 reviews for vol. xxiii. Of the papers in the 1937 volume, 41 were by members of the Association and 26 by non-members. The papers may be roughly classified as follows: plant physiology and non-parasitic diseases 13, mycology and fungus diseases 10, bacterial diseases 2, virus diseases 7, applied entomology 17, plant protection 11, microbiology of soil, etc. 4, general 2, apparatus 1.

The several parts of vol. xxiv of the *Annals of Applied Biology* were published on the following dates:

Part 1. 4 March.
Part 2. 26 May.
Part 3. 17 August.
Part 4. 17 November.

W. P. K. FINDLAY
G. FOX-WILSON } *Hon. Secretaries.*

REPORT OF THE HON. TREASURER FOR THE YEAR ENDING 31 DECEMBER 1937

During the year ending 31 December 1937 subscriptions and entrance fees, including arrears paid in, amounted to £337. 7*s.* 0*d.*, almost exactly the same amount as last year. Income from the sale of the current volume of the *Annals of Applied Biology* and from reprints amounted to £809. 5*s.* 0*d.*, an increase over last year of £108, mostly attributable to increased reprint sales. The size of the *Annals* was again increased as compared with last year and amounted to 940 pages as against 728 pages in 1934, and the cost of producing it rose to £1330, an increase of £120 over last year's figures.

On the whole year there has been an excess of expenditure over income of £100. 6*s.* 2*d.* as against £101 last year, the increased cost of the *Annals* being compensated by a larger sale of reprints and the greater value of the stock.

The financial position of the Association is sound, with an excess of assets over liabilities of £852; but if we are to maintain the *Annals* at its present size it is essential that we increase our membership. At present the number of new members just compensates those lost through death and resignation and it is very desirable that every opportunity should be taken of obtaining additional members.

J. HENDERSON SMITH,
Hon. Treasurer.

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY* INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1937 *Cr.*

Expenditure

	£ s. d.	£ s. d.
To Estimated Value of Stock at 1 January 1937		130 14 6
To Cambridge University Press		1330 10 1
To Copies bought in		13 10 0
		£1474 14 7

Income

	£ s. d.	£ s. d.
By Sales—Current Volume		600 16 6
By Sales—Back Volumes, Parts and Sets		66 16 3
By Sales of Reprints		208 8 6
By Estimated Value of Stock at 31 December 1937		189 19 0
By Balance, carried down		408 14 4
		£1474 14 7

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1937 *Cr.*

Expenditure

	£ s. d.	£ s. d.
To *Annals of Applied Biology*, balance brought down		408 14 4
To Printing and Stationery		13 12 4
To Postages and Cheque Stamps		8 16 4
To Honorariums		6 3 0
To Subscription—Parliamentary Science Committee		10 10 0
To Sundry Out-of-Pocket Expenses of Secretaries and Treasurer		12 3 7
To Audit Fee Reserve		4 4 0
		£464 3 7

Income

	£ s. d.	£ s. d.
By Members' Subscriptions:		
Arrears	23 15 0	
Entrance Fees	7 7 0	
Current	306 5 0	
		337 7 0
By Interest on National Savings Certificates and Bank Deposit		26 10 5
By Balance, being Excess of Expenditure over Income for the year		100 6 2
		£464 3 7

BALANCE SHEET 31 DECEMBER 1937

Liabilities and Surplus

	£ s. d.	£ s. d.
Sundry Creditors:		
Cambridge University Press	467 18 10	
Audit Fee Reserve	4 4 0	
Sundry Expenses	17 16 8	
		489 19 6
Subscriptions and Entrance Fees paid in advance		12 6 0
Excess of Assets over Liabilities:		
As Balance Sheet of 31 December 1936	953 4 9	
Less: Balance of Income and Expenditure Account for 1937	100 6 2	
		852 18 7
		£1355 4 1

Assets

	£ s. d.	£ s. d.
Cash:		
At Bank on Current Account	74 0 1	
At Bank on Deposit Account	250 0 0	
		324 0 1
Debtors for Subscriptions, 2 years or less in arrear and considered good		60 0 0
500 National Savings Certificates		781 5 0
Stock of *Annals of Applied Biology* at estimated value		189 19 0
		£1355 4 1

J. HENDERSON SMITH, *Hon. Treasurer*.

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

(Signed) H. J. COX & CO. *Incorporated Accountants* — *Auditors.*

HARPENDEN, 8 *February* 1938.